PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 28 November 15, 1942 Number 11
Copyright 1942 by the National Academy of Sciences

SOME FACTORS AFFECTING THE TOXICITY OF CULTURES OF SHIGELLA DYSENTERIAE

By René J. Dubos,* Henry D. Hoberman† and Cynthia Pierce

Harvard Schools of Medicine and Public Health

Communicated October 15, 1942

Cultures of *Shigella dysenteriae* are extremely toxic to man and experimental animals; it is likely that this toxicity is of great significance in determining the symptomatology and pathology of Shiga dysentery. Many conflicting views have been voiced to account for it. Some authors, for instance, feel that all the toxic manifestations are due to one single substance.[1] Others, on the contrary, believe in the existence of two independent toxins [2–8]: (*a*) an endotoxin, which exhibits a special affinity for the intestinal tract, is present only in the smooth variants of the organism, is associated chemically with the specific somatic polysaccharide and is resistant to heat and to proteolytic enzymes; (*b*) an exotoxin with neurotropic affinity, which is present both in the rough and smooth variants, is of protein nature and is inactivated by heat and trypsin. It has also been claimed that aerobic incubation favors the production of the exo-neurotoxin whereas only the endotoxin is produced under anaerobic conditions.[2]

It is obvious that, before any final claim can be made concerning the nature and pathological activity of the toxic components of the Shiga bacillus, methods have to be devised for the production in large quantities and for the purification of these substances. In view of the practical importance of this problem for the eventual development of immunization procedures, we are reporting at the present time some of our preliminary observations which may serve to define better the optimal conditions for the production of toxin and the properties of at least one of the toxic fractions.

Effect of Cultural Conditions on the Toxicity of Cultures of Shigella dysenteriae.—All experiments were performed with strain 32158 of *Shigella dysenteriae* which was obtained through the courtesy of Miss M. Coleman of the New York State Department of Health. Two phase variants were used: (1) the smooth form (*s*) which gave typical agglutination in standard antisera, and (2) a rough variant (R_{12}) which was isolated from an old stock

culture. The organisms grown in liquid media were collected by centrifugation or filtration (in the case of the *R* variant); the agar cultures were suspended in distilled water and also centrifuged. It was observed that in the case of young cultures at least (24 to 48 hours old, for instance), only a very small percentage of the total toxicity of the whole culture was lost in the culture supernatant or cell washings; in other words, the toxic components of young cultures seemed to be bound to the cell structure, a fact already observed by others.[9]

The dry weights of the cultures were obtained by desiccation over phosphorus pentoxide *in vacuo*, or by treatment with acetone ether; it was found that these methods of desiccation did not alter the toxicity of the original material.

A number of different toxicity tests were used: minimal lethal dose for rabbits and for white mice (20 grams), production of the Shwartzman reaction in rabbits,[10] production of the ocular reaction in rabbits,[11] etc. Rabbits were found to be much more susceptible than mice; for reasons of economy, however, most titrations of toxicity were based on determination of the minimum lethal dose for mice, and only the results of these tests will be reported at the present time.

The effect of cultural conditions on toxicity is well illustrated by the following experiment. Cultures of rough and smooth variants of Shiga bacillus were grown at 37° for 18 hours in meat infusion peptone broth, (the layer of fluid being 10 cm. thick), or on meat infusion peptone agar. The washed cells were desiccated *in vacuo* over phosphorus pentoxide, resuspended in 0.05 *M* phosphate buffer at pH 7.2, heated at 60°C. for 10 minutes, and injected intraperitoneally into white mice. The results presented in table 1-A indicate that in the case of both the rough and smooth variants, 0.15 mg. was the minimal lethal dose when the agar cultures were used, and 1.5–2.0 mg. with the broth cultures.

Aqueous extracts of agar were added to meat infusion peptone broth to determine whether there is present in agar a water-soluble component which favors toxin production; no increase in toxicity of the broth culture, however, resulted from this treatment.

It appeared possible that the greater production of toxin on agar media was due to the fact that the agar gel permits surface growth and therefore increases aerobic metabolism. To test this hypothesis, media were prepared in which the solid surface was supplied by silica gel[12] instead of agar. To the silica gel were added 0.05 *M* phosphate buffer at pH 7.4, 1.0 per cent peptone and 1.0 per cent meat extract. The medium was poured into petri dishes, some of the plates receiving 1.0 per cent dextrose in addition to the other components of the medium. The plates were inoculated with rough or smooth variants of Shiga cultures; after 18 hours' incubation, the growth (which was less abundant than on agar) was collected from the

TABLE 1

TOXICITY OF DESICCATED CELLS OF *R* AND *S Shigella dysenteriae* GROWN UNDER DIFFERENT CONDITIONS

CULTURE	MEDIUM	NUMBER OF MICE SURVIVING I.P. INJECTION OF FOLLOWING AMOUNTS OF CELLS (MG. DRY WEIGHT)			
	1-A				
		1.5	1.0	0.3	0.1
R	Meat infusion peptone agar	0/3[a]	0/3	0/3	1/3
R	Meat infusion peptone broth	2/3	2/3	3/3	3/3
S	Meat infusion peptone agar	0/3	0/3	0/3	0/3
S	Meat infusion peptone broth	2/3	3/3	3/3	3/3
	1-B				
		1.0	0.5	0.25	0.1
R	Nutrient silica gel	0/1	0/2	0/3	0/2
R	Nutrient silica gel + 1% glucose	0/1	0/2	2/3	3/3
S	Nutrient silica gel	...	0/3	0/3	0/3
S	Nutrient silica gel + 1% glucose	...	0/3	2/3	3/3
	1-C				
		1.5	0.75	0.45	0.15
R	Peptone broth mechanically shaken (total yield 600 mg./liter)	0/3	0/3	1/3	2/3
R	Anaerobic peptone broth (total yield 200 mg./liter)	1/3	3/3	3/3	3/3
	1-D				
		1.5	0.75	0.30	0.15
R	Peptone broth mechanically shaken (total yield 750 mg./liter)	...	0/3	2/3	3/3
R	Thioglycollate peptone broth (total yield 420 mg./liter)	3/3	3/3	..	..

[a] The denominator indicates the number of mice used in the test. The numerator indicates the number of mice surviving after 10 days.

silica gel surface, washed with water, desiccated with acetone ether, resuspended in neutral phosphate buffer, heated at 60°C. for 10 minutes and injected intraperitoneally into white mice. The results presented in table 1-B indicate that, in the case of both the rough and smooth variants, the cells grown on silica gel medium not containing glucose were extremely toxic, even more toxic than the cultures grown on agar media. Addition of glucose to the medium markedly reduced toxicity.

Since surface growth gives rise to cells much more toxic than the cells grown in liquid media, an attempt was made to increase oxidative conditions within the broth by bubbling oxygen during the whole course of incubation; these conditions brought about some increase in toxicity, but the

results were not striking enough to warrant further description at the present time.

In other experiments, the inoculated medium was violently agitated on a shaking machine during the whole period of growth. It was found that, not only did the shaking markedly increase the total yield of cells, but that the toxicity per milligram of dry weight of cell was also greatly increased. These facts are illustrated in the two following experiments.

A medium containing 0.05 *M* phosphate at pH 6.9, 1.0 per cent peptone (Difco tryptone) and 1.0 per cent glucose was inoculated with a rough variant of *Shigella dysenteriae*. Half of the medium was incubated in an aerobic jar;[13] the other half was violently agitated on a shaking machine during the whole incubation period. The cells were collected by centrifugation after acidification at pH 4.5 (a reaction at which none of the broth constituents are precipitated), washed with water and desiccated with acetone ether. The total yields were approximately 600 mg. per liter in the case of the aerobic culture, and only 200 mg. in the case of the anaerobic culture. The cells were resuspended in neutral phosphate buffer, heated at 60°C. for 15 minutes and injected intraperitoneally into white mice for a determination of comparative toxicity (table 1-C).

In another experiment anaerobic conditions of incubation were obtained by the addition of 0.05 per cent of sodium thioglycollate to the medium, and aerobic conditions secured by constant agitation. The medium consisted of 1.0 per cent tryptone (Difco), 1.0 per cent glucose, 0.05 *M* phosphate at pH 7.8, and was inoculated with a rough variant of Shiga bacillus (table 1-D).

The results presented in tables 1-C and 1-D indicate clearly that anaerobic incubation gives yields of cells very much smaller than those obtained under conditions of aerobic agitation during growth (750 mg. per liter versus 420). The toxicity of the cells grown under aerobic conditions is also much greater per milligram dry weight than that of cells obtained by anaerobic incubation.

Separation of a Toxic Component from Cultures of a Rough Variant of Shigella dysenteriae.—Large amounts of culture of the variant form R_{12} of the *Shigella dysenteriae* were grown on meat infusion peptone agar at 37°C. for 18 hours; the cells were washed in water and desiccated *in vacuo* over phosphorus pentoxide. The average yield was approximately 20 mg. of washed dried cells per agar plate (8 cm. diameter); 0.15 mg. of dried cells (heated at 60°C. for 15 minutes in 0.05 *M* neutral phosphate buffer) was the minimal lethal dose for mice. The bacterial component responsible for the toxicity of the culture exhibits the following properties: (1) it is only slowly inactivated by heat at neutral and at acid reactions, but is very rapidly destroyed at alkaline pH; (2) it is completely resistant to proteolytic enzymes (crude trypsin, pepsin, papain, mold and bacterial en-

zymes); (3) it is precipitated by two-thirds saturation with ammonium sulfate, and by 33 to 50 per cent acetone; (4) it is precipitated at acid pH (pH 3.0 to 4.0); (5) it can be obtained in solution in 0.05 *M* dibasic phosphate from heat-killed cultures digested with trypsin; (6) the addition of papain to a solution of the toxic material causes the precipitation of an inactive fraction (probably nucleic acid), whereas the active material remains in solution; it can be precipitated again from the papain solution by addition of acid; (7) the active principle does not dialyze through cellophane membranes.

By making use of these different properties, we have obtained fractions of such activity that 3.0 micrograms injected intraperitoneally into white mice, or intravenously into rabbits, regularly causes death within 3 to 4 days; death of mice was also observed with amounts as small as 1.3 microgram. The material is stable and retains its activity unaltered for several weeks when kept in neutral solution at 5°C.

Conclusions.—It has been shown that conditions which favor aerobic metabolism (growth on agar or on silica gels, or in broth violently agitated during incubation) greatly increase the yield of *S. dysenteriae* cultures and the toxicity of the cells (measured in terms of dry weight). For instance, 1.5 mg. of dried cells grown in an anaerobic jar failed to kill any mice, whereas 0.1 mg. of cells grown on silica gel plates killed all mice (smaller amounts were not tried). The addition of glucose to silica gel medium caused a definite decrease in the toxicity of the cells; this may be due to the fact that, even when growth takes place under aerobic conditions, the presence of glucose permits some anaerobic metabolism.

The effect of environmental conditions of growth on the yield of organisms and on the yield of toxin was the same for rough and for smooth variants of *Shigella dysenteriae.* In all cases also, the largest percentage of total toxicity of the whole culture was found to be bound to the bacteria bodies and to be released in solution only as a result of autolysis.

The toxic factor is resistant to proteolytic enzymes; it can be obtained in the form of a water-soluble fraction which is stable and of which 1.0 to 3.0 micrograms cause the death of mice and rabbits within 72 hours.

* Preliminary experiments were carried out in the laboratories of the Hospital of the Rockefeller Institute, New York.

† Fellow in Medical Sciences of the National Research Council.

[1] Waaler, E., *Jour. Exper. Med.*, **63**, 1–16 (1936).

[2] Olitzki, L., and Leibowitz, J., *Brit. Jour. Exper. Path.*, **16**, 523–531 (1935).

[3] Boivin, A., and Mesrobeanu, L., *Compt. Rend. Soc. Biol.*, **124**, 439, 442, 1078, 1092 (1937).

[4] Boivin, A., *Ibid.*, **133**, 252–255 (1940).

[5] Haas, R., *Ztschr. Immunitäts. exper. Therap.*, **91**, 254–272 (1937).

[6] Haas, R., *Ibid.*, **99**, 121 (1940).

[7] Istrati, Gh., *Compt. Rend. Soc. Biol.*, **129**, 1010–1013 (1938).
[8] Wagner-Jauregg, Th., *Centralbl. f. Bakt.*, **144**, I. Orig., 31–32 (1939).
[9] Okell, C. C., and Blake, A. V., *Jour. Path. & Bact.*, **33**, 57–63 (1930).
[10] Shwartzman, G., "Phenomenon of Local Tissue Reactivity," N. Y., 461 pp. (1937).
[11] Ajo, C., *Proc. Soc. Exper. Biol. & Med.*, **47**, 500–501 (1941).
[12] Sterges, A. J., *Jour. Bact.*, **43**, 317–327 (1942); *Ibid.*, **44**, 138 (1942).
[13] Mueller, J. H., and Miller, P. A., *Ibid.*, **41**, 301–303 (1941).

THE FUSION OF BROKEN ENDS OF CHROMOSOMES FOLLOWING NUCLEAR FUSION

By Barbara McClintock

Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor

Communicated September 22, 1942

When, through radiation or other causes, chromosomes are broken within a single nucleus, 2-by-2 fusions may occur between the broken ends. These fusions may lead to rearrangements of parts of the chromatin complement, giving rise to various chromosomal aberrations which are detected as reciprocal translocations, inversions, deficiencies, etc. Since, in the well-investigated cases, the breakages occurred within a single nucleus, the conditions that lead to fusions of broken ends could not easily be ascertained. The following questions have been asked: (1) Must two or more chromosomes be in intimate contact at the time of breakage in order that fusions may occur? (2) If no intimate contact is necessary at the time of breakage, are the broken ends "unsaturated," that is, capable of fusion with any other unsaturated broken end? (3) If question (2) can be answered in the affirmative, what forces are involved which lead to the contact and subsequent fusions of the two unsaturated broken ends? Likewise, (4) how long will these broken ends remain unsaturated, i.e., capable of fusion?

Questions (1) and (2) could be answered if the following conditions were present: Assume that fusion occurs between two nuclei each of which possesses one chromosome, one end of which has been broken. Each nucleus will then have a single broken end. When these nuclei fuse and their chromosomes intermix within a single nucleus, the chromosome with a broken end contributed by one nucleus could fuse with the chromosome with a broken end contributed by the second nucleus. The chromosome fusion should occur between these two broken ends. This experiment may easily be conducted in maize. The two nuclei that fuse can be the male and the female gametes, respectively. The method of obtaining

gametes having a chromosome with a single "unsaturated" broken end has been reported previously.[1] This method may be briefly summarized. Plants were obtained which possessed one normal chromosome 9 and one chromosome 9 with a duplication of the short arm. This duplicated arm extended beyond the normal short arm, the serial order of parts within the duplicated segment being the reverse of that of the normal short arm. When the duplicated segment is involved in crossing-over, a dicentric chromatid may be produced which is the equivalent of two chromosomes 9 attached at the ends of their short arms. Breakage of this dicentric chromatid during a meiotic anaphase results in the entry into a spore nucleus of a chromatid with a single broken end. Fusion then occurs at the position of breakage between the two sister halves of this broken chromatid, forming a new dicentric chromatid. This, in turn, produces a bridge configuration in the first gametophytic division, as the two centromeres of the dicentric chromatid pass to opposite poles in the anaphase figure. Again, a broken chromatid enters each telophase nucleus. In each nucleus, fusion again occurs between the two sister halves of this broken chromatid at the position of this latter breakage. This *chromatid* type of breakage-fusion-bridge cycle continues in successive gametophytic mitoses. Therefore, all the nuclei of the fully developed male or female gametophyte will possess one chromosome with a single broken end. Following fertilization, two nuclei from the female gametophyte and one from the male gametophyte fuse to form the primary endosperm nucleus. If the nuclei of one of the gametophytes possesses such a chromosome carrying dominant genes in the arm with the broken end, and if the other gametophyte possesses a normal, non-broken chromosome carrying the recessive alleles, variegation for these genes will be apparent in the fully developed endosperm. This is because the chromosomes with the broken ends continue the breakage-fusion-bridge cycle in successive nuclear divisions during the development of the endosperm. Following various non-median breakages of the bridge configurations, the dominant genes may be deleted from some nuclei and duplicated in the sister nuclei. Continued repetition of this type of breakage during the development of the endosperm produces a conspicuous variegation pattern. The behavior of the chromosome with the broken end in the sporophytic tissues of these kernels is entirely different. The chromatid type of breakage-fusion-bridge cycle ceases when the zygote is formed. The newly broken end "heals." Following this healing, no further fusions occur. The broken end is as stable in its subsequent behavior as any normal chromosome end.

There is no reason to suspect that the condition of those chromosomes in the gamete nuclei that participate in endosperm fusions differ from those participating in zygotic fusions. However, in one case, the broken end remains unsaturated, and in the other case the broken end heals. It is

reasonable to believe, therefore, that the healing process occurs subsequent to zygotic fusions and not before. One may tentatively assume that a chromosome end, broken in the pre-gametic division, is unsaturated at the time of zygotic fusion. If each gamete contributes a chromosome with an unsaturated broken end, one could expect fusion to occur between these two broken ends. The reasoning behind this expectation is based on the behavior of ring-shaped chromosomes in sporophytic tissues.[2] Ring-shaped chromosomes are frequently broken during mitotic anaphases. Following such breakage, the telophase nuclei receive a chromosome both ends of which are broken. Fusion may then occur between these unsaturated broken ends, reëstablishing the ring-shape. This is a chromosome fusion, not a chromatid fusion. This behavior has suggested that fusion of unsaturated broken ends in sporophytic tissues may be chromosomal in contrast to gametophytic and endosperm tissues where chromatid fusions may occur. The experiment to be described furnishes proof of the correctness of these assumptions.

To test whether the broken ends are unsaturated in the gamete nuclei, two such ends were introduced into the zygote nucleus, one contributed by the male gamete and one by the female gamete. Detection of kernels whose zygote nuclei had received such chromosomes was accomplished by introducing contrasting endosperm markers carried by the chromosomes with the broken ends (*i*, aleurone color, and *wx*, waxy starch, located in the short arm of one parental chromosome 9 and the alleles *I*, inhibitor of aleurone color, and *Wx*, normal starch, carried by the other parental chromosome 9). The endosperms of those kernels that receive a broken chromosome 9 from each parent should show a very distinctive type of variegation. All three broken chromosomes 9 would undergo the break-fusion-bridge cycle. This would lead to variegation for *I-i* and *Wx-wx*, variegation for depth of color in the *i* regions due to multiplication of the number of *i* genes, and scarred and pitted regions in both the *I* and *i* sectors due to the presence of cells which are homozygous deficient for segments of the short arm of chromosome 9.[3]

Plants heterozygous for the duplication chromosome 9 and homozygous for *i wx* were crossed by plants heterozygous for the duplication and homozygous for *I* and *Wx*. Three types of kernels, with respect to endosperm characters, should be produced; (1) *I Wx*, non-variegated kernels following fusion of a nucleus carrying a non-broken *I Wx* chromosome with nuclei carrying either a non-broken or a broken *i wx* chromosome; (2) kernels variegated for *I-i* and *Wx-wx* following fusion of a nucleus carrying a broken *I Wx* chromosome with nuclei carrying a non-broken *i wx* chromosome; (3) kernels resulting from the fusion of a nucleus carrying a broken *I Wx* chromosome with nuclei carrying a broken *i wx* chromosome. As stated above, the endosperm character of this latter type of kernel could be

anticipated. When observations were made of the kernels resulting from this cross, these latter kernels were very conspicuous. From a total of 18,243 kernels examined, 20 possessing an embryo were of this latter type. More of this type were present but they were germless. These 20 kernels were germinated to determine what had happened to the two broken chromosomes 9 which had entered the zygote. If both broken ends had healed without fusion, normal-appearing plants would be expected to arise from these kernels. If the two broken ends had fused, a dicentric chromosome would have been produced. It would be composed of the chromosome 9 contributed by the male gamete and the chromosome 9 contributed by the female gamete, with their short arms fused end-to-end. When this dicentric chromosome divided and when the two centromeres of each chromatid passed to opposite poles at a mitotic anaphase, two contiguous bridges should be formed. Following breakage of these two bridges, two chromosomes, each with a freshly broken end, should enter each sister telophase nucleus. As stated previously, one could expect fusions to occur between the broken ends of these two chromosomes in each sister telophase nucleus. This would reëstablish the dicentric condition, for again the two chromosomes 9 would be joined to form one chromosome with two centromeres. Repeated anaphase bridge configurations should be expected in subsequent divisions following this *chromosomal* type of breakage-fusion-bridge cycle. The cells of a plant possessing such a dicentric chromosome undergoing this behavior should be composed of various types of homozygous and heterozygous duplications and deficiencies of the short arm of chromosome 9, following repeated non-median breaks in the anaphase bridges. Consequently, these plants should be conspicuously modified in appearance, because of the variation in degree of duplication or deficiency in the many nuclei of the plant.

In the seedling stage, 10 of the 20 plants arising from the kernels classified as having received a broken chromosome 9 from each parent were obviously of the type expected if a dicentric chromosome were present. The presence of the dicentric chromosome was confirmed by examination of the division figures in the young roots, where nearly half of the observed anaphase figures showed two contiguous bridges derived from a dicentric chromosome. Nine of the remaining plants were normal in appearance, and one plant was normal in morphological growth but pale yellow[4] in color and died in the seedling stage. No bridges were observed in the roots of these latter 10 plants.

Due to aberrant growth and death of many cells, 5 of the plants with a dicentric chromosome died in the seedling stage. The remaining 5 plants continued to grow. In all 5 plants, as growth continued, sectors of tissue developed which showed no aberrant growth patterns. These sectors were quite normal in appearance. Gradually, these recovered sectors gained the

ascendency in growth until most of the plant was normal in appearance. In one plant, 3 normal side shoots developed from the base of a very aberrant main shoot which was obviously dying. Root tips were taken at various times from all of the 5 plants that had survived the seedling stage. In all cases, vigorous growth of some side branches of the root system was noted. Examination of division figures in these roots no longer showed any dicentric bridges. The examined cells possessed the normal chromosome number of 20, instead of the 18 monocentric chromosome plus 1 dicentric chromosome previously observed in the younger roots. Sporocytes for examination of the chromosomes at pachytene were obtained from two of the three recovered shoots of one plant and from the recovered main shoots of the four other plants. In all cases, 10 bivalent chromosomes were present, one of which was a bivalent chromosome 9. The two chromosomes 9 were not fused at the ends of their short arms. In most cases, the composition of the short arm of each member of the bivalent was greatly modified, although in each tassel sample the two chromosomes 9 maintained their respective morphologies in all examined cells. Of the 12 chromosomes 9 examined from these six samples, no two were alike. The composition of the short arms of the chromosomes 9 in the two recovered branches from one plant originally possessing a dicentric chromosome was entirely different. In each case, it was apparent that the cells of the examined part of the tassel had originated from one individual cell whose cell ancestors had previously been undergoing the chromosomal type of breakage-fusion-bridge cycle involving the original dicentric chromosome 9. This could be determined in each case by the comparative morphologies of the short arms of the two members of the bivalent. In several cases it was possible to determine the minimum number of fusions, bridges and breaks that must have occurred before "healing" of the two broken ends had taken place in the nucleus of the particular cell that gave rise to the recovered sector. The factors involved in the process of healing of two such broken ends within a single nucleus are still undetermined.

The pachytene chromosomes were examined in the surviving 9 of the 10 plants which were normal in morphological growth from the earliest seedling stage and which showed no bridges in the earliest roots. This examination showed that 4 of these plants had received a broken chromosome 9 from each parent. However, morphological analysis of the short arms of the two chromosomes 9 in each case gave no indication that fusions had ever occurred between their broken ends. In one plant, one parent had contributed a broken chromosome 9, but it was not possible to determine whether the other parent had contributed a broken chromosome 9. In the remaining 4 plants, each parent had contributed a broken chromosome 9. However, the broken end of one chromosome 9 had fused with a broken end of a chromosome other than that of the chromosome 9 introduced by the

second gamete. In each case, the broken end of the second chromosome 9 had no unsaturated end with which it could unite. As expected, this single broken end thereafter healed.

Conclusions.—The experiments outlined allow some specific answers to be given to the questions presented in the first paragraph of this paper. Question (1) may be answered in the negative. Two chromosomes do not have to be in contact at the time of breakage in order that fusions may occur between their broken ends. This was shown by the fusion that occurred in the zygote or in an early embryonic nucleus between a broken end of chromosome 9 contributed by the male gamete and a broken end of the chromosome 9 contributed by the female gamete. Question (2) may be answered in the affirmative. This was shown by the fusion of these two chromosomes, which produced a dicentric chromosome, and the subsequent behavior of this dicentric chromosome which, for some time, followed the chromosomal type of breakage-fusion-bridge cycle. Question (3) cannot be answered directly from the present observations. Nevertheless, the observations imply that some force exists which accounts for the fusion of unsaturated broken ends of chromosomes. Question (4) likewise cannot be answered directly. Nevertheless, it is certain that the unsaturated state does not persist indefinitely. An unsaturated broken end will become saturated or healed and incapable of fusions when only one such broken end is present in sporophytic tissues;[1] or, as shown in this report, two such broken ends may heal without fusions even when these two ends are present in the same nucleus.

[1] McClintock, B., *Genetics*, **26**, 234–282 (1941).
[2] McClintock, B., *Ibid.*, **23**, 315–376 (1938).
[3] McClintock, B. (unpublished).
[4] This mutant type appears when a plant is homozygous deficient for a small terminal segment of the short arm of chromosome 9.

NON-RANDOM UNCOILING IN HETEROBRACHIAL CHROMOSOMES

BY A. H. SPARROW*

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated October 2, 1942

In theories regarding the mechanics of chromosome spiralization much depends upon whether there is a definite (inherent?) pattern of coiling or whether the direction of coiling is purely a matter of chance, determined independently in the two arms of the chromosome. Reversals of direction

may occur at the kinetochore (interbrachial) or interstitially within an arm (intrabrachial). The present paper will be concerned only with interbrachial reversals and will deal with chromatid relational coiling proximal to the kinetochore, regardless of the direction of twisting in more distal regions.

The direction of relational coiling has been reported to be random in the two arms of a chromosome in *Trillium*,[1, 2] *Vicia*,[1] *Hyacinthus*[3] and *Allium*[4] and non-random in *Tradescantia*[5] and in the *B* chromosome of *Trillium*.[2] Koshy states that reversals of direction always occur at the kinetochore in *Allium*[6] and *Aloe*[7] and considers them essential to relational untwisting. Darlington[8] found that the direction of relational coiling "shows no consistency" in *Spironema*, *Crocus* and *Pushkinia*, is "sometimes inconsistent" in particular arms in *Nomocharis* and is "usually consistent in each arm of all chromosomes in *Fritillaria* and constant for the identifiable *M* chromosomes."

In *Trillium* the major coil of the first meiotic division persists throughout second division and appears as a plectonemic relic coil at prophase of the first division of the microspore.[2, 9] The relational coil is derived directly from the relic coil and, therefore, should show the same randomness in direction of coiling as does the major coil of meiosis.[10, 11, 12, 13] However, in an analysis of chromatid relational coiling in *Trillium* microspores Sparrow, Huskins and Wilson found that coiling was not random in the *B* chromosome, although it was random in the *C*, *D* and *E* chromosomes. (The kinetochore in the *A* chromosome is subterminal.)

This brief review of the literature on relational coiling indicates much disagreement. The present paper is an attempt to reconcile some of the apparent discrepancies by a detailed study of relational coiling in a plant for which both random and non-random coiling have been reported.

Methods.—Fresh anther smears of *Trillium grandiflorum* Salisb. were stained with iron aceto-carmine, pressed and sealed in the usual manner. Some of the slides were made permanent but a few "temporary" mounts have been kept in cold storage for nearly three years and are still usable. Camera lucida drawings of whole complements were made from nuclei in which the chromosomes were well separated. The number of relational twists present in an arm decreases as prophase advances and by metaphase none or only a few remain. Early stages are difficult to analyze and late stages have too many untwisted arms. Consequently the majority of the cells studied were late prophases in which most of the arms had two or more twists. Their mean total chromatid length per complement was 158.1 μ with a Standard Error of 4.3 μ. (See previous publications[2, 9] for illustrations and data on chromatid length changes.)

Results.—The direction of relational twists proximal to the attachment region of the *B*, *C*, *D* and *E* chromosomes are given in table 1 for a total

of 89 cells. Table 2 gives the number of chromosomes with reversals (R.L), the number without reversals (R.R and L.L) and in the third column the remainder. The latter group includes those chromosomes in which at least one arm had lost all its relational twists plus three cases in which it was not possible to determine the direction of coiling of the most proximal twists.

If the direction of coiling were independent in the two arms of a chromosome then the number of chromosomes with interbrachial reversals should be approximately equal to the number without them. For the *E* chromosome the numbers do not deviate significantly from the theoretically expected 1:1 ratio ($\chi^2 = 1.026$), but the *B*, *C* and *D* chromosomes all show a statistically significant deviation (see table 2 for χ^2 and *P* values). There is an excess of chromosomes without reversals. Apparently, therefore, the direction or relational coiling is random across the kinetochore in the *E* chromosome but not in the *B*, *C* and *D* chromosomes, or at least so it appears when only chromosomes with both arms still relationally twisted are considered. When all chromosomes are considered a different interpretation is possible.

TABLE 1

Direction of Relational Twists Proximal to the Kinetochore in the *B*, *C*, *D* and *E* Chromosomes of 89 Microspores of *Trillium grandiflorum*

(R = right, L = left, U = untwisted, . = kinetochore)

CHROMOSOME	R.R	R.L	L.L	U.R	U.L	U.U	?
B	23	17	12	10	23	4	0
C	16	10	9	21	20	12	1
D	18	20	22	13	7	8	1
E	11	44	24	5	4	0	1
—	—	—	—	—	—	—	—
Total	68	91	67	49	54	24	3

Relational twists are not eliminated from all chromosomes simultaneously. The shorter arms having fewer relational twists untwist sooner, on the average, than the longer arms. Thus the *B*, *C* and *D*, respectively, had 37, 53 and 28 chromosomes with one or both arms untwisted while the *E* had only nine (table 2). Since all the chromosomes are from the same 89 cells any difference in number of relational twists (or lack of them) must be due to morphological or structural differences in the chromosomes themselves. The most obvious difference is in the position of the kinetochore and the relative lengths of the chromosome arms. The *E* chromosome, with median kinetochore and both arms long, untwists, on the average, later than the chromosomes with shorter arms and sub-median kinetochore. Consequently the *E* chromosome data most nearly represent the initial relational twisting of a sample of cells in which untwisting has not yet begun.

Since a 1:1 ratio of chromosomes with interbrachial reversals to those without is expected for all chromosomes, then the *B*, *C* and *D* chromosomes show a significantly lower number of chromosomes without reversals. On the basis of selective or non-random untwisting this means that more chromosomes with reversals than without have untwisted. The remaining chromosomes no longer can be considered as representative of an unselected sample.

The limits of a 1:1 ratio can be calculated from the formula $\sigma = \sqrt{npq}$. When $n = 89$ and p and $q = 0.5$ the value of σ is 4.72. The limits of the 1:1 ratio are then $44.5 \pm 2\sigma$ or 35.1 and 53.9. Of the 89 *B* chromosomes 37 have one or both arms untwisted and hence cannot be classified as to reversals at the kinetochore. However, if 19 of them had previously had reversals then 36 of the total of 89 would have reversed direction leaving 53 without reversals. These numbers are just within the limits of the 1:1 ratio. In order to approximate more closely to a perfect 1:1 ratio more than half of the untwisted chromosomes must be considered to have had an interbrachial reversal. The same is true for the *C* and *D* chromosomes. It is therefore suggested that the apparent excess of chromosomes without interbrachial reversals is the result of earlier untwisting in at least one arm of chromosomes with interbrachial reversals. It thus appears that uncoiling does not occur entirely independently in the two arms of a heterobrachial chromosome, but is affected by the presence or absence of interbrachial reversals.

TABLE 2

Chromosomes with and without Reversals at the Kinetochore and the χ^2 and *P* Values Corresponding to the Observed Deviation from the Expected 1 : 1 Ratio

CHROMOSOME	NUMBER WITH REVERSALS	NUMBER WITHOUT REVERSALS	NUMBER NOT DETERMINED	χ^2	*P*
B	17	35	37	6.230	<0.02
C	10	25	54	6.428	<0.02
D	20	40	29	6.666	<0.01
E	44	35	10	1.026	>0.3
Total	91	135	130	$\Sigma\chi^2 = 20.350$	<0.01

In chromosomes devoid of reversals untwisting can occur only at the distal ends. Intrabrachial reversals allow direct cancellation and with interbrachial reversals untwisting may occur also at the proximal ends. If the kinetochore is undivided direct cancellation cannot take place at the interbrachial reversal but essentially the same result will be obtained if the kinetochore rotates. This would be expected to happen only when the untwisting forces from the two arms are augmentive as they would be when the two arms are twisted in opposite directions.

Intrabrachial reversals were previously shown to play a part in the

elimination of relational coiling.[2] It now appears that both intra- and interbrachial reversals are instrumental in reducing the number of chromatid relational twists during prophase uncoiling. Further, if interbrachial reversals in heterobrachial chromosomes lead to non-random uncoiling it is apparent that non-randomness in direction of relational coiling does not necessarily mean that the spirals were non-random at the time of their inception. It seems probable, therefore, that some of the previously reported non-randomness in direction of relational coiling has also been the result of the alteration of the original 1:1 ratio by disproportionate untwisting in chromosomes with interbrachial reversals. The extent of the deviation from the expected ratio would presumably increase in accordance with the amount of untwisting. A sample of late prophases would thus be expected to have a greater deviation than a sample composed largely of earlier prophases. Considerable variation from sample to sample should, therefore, be expected.

Summary.—Four chromosomes from each of 89 microspores of *Trillium grandiflorum* Salisb. were analyzed to determine whether or not the direction of chromatid relational coiling was random across the kinetochore. The direction of coiling was random in the *E* chromosomes but apparently non-random in the *B*, *C* and *D* chromosomes. In the latter the excess of chromosomes without interbrachial reversals is not to be regarded as an initial non-randomness attributable to inherent or mechanical factors. The evidence is taken to indicate not that the direction of coiling was non-random at its inception but that the non-randomness arose as a result of disproportionate untwisting in chromosomes with interbrachial reversals. In brief, the apparent non-random coiling is in reality a non-random untwisting. After complete untwisting in some of the chromosomes arms the direction of coiling across the kinetochore in the remaining heterobrachial chromosomes then shows a significant deviation from the expected randomness.

* National Research Fellow.

[1] Sax, K., *Genetics*, **21**, 324–338 (1936).
[2] Sparrow, A. H., Huskins, C. L., and Wilson, G. B., *Can. Jour. Res., C*, **19**, 323–350 (1941).
[3] Upcott, M., *Cytologia*, **8**, 398–407 (1938).
[4] Husted, L., *Ibid.*, **8**, 368–376 (1938).
[5] Husted, L., *Genetics*, **21**, 537–553 (1936).
[6] Koshy, T. K., *Jour. Roy. Mic. Soc.* (Ser. 3), **53**, 299–318 (1933).
[7] Koshy, T. K., *Ann. Bot.* (N. S.), **1**, 43–58 (1937).
[8] Darlington, C. D., *Cytologia*, **7**, 248–255 (1936).
[9] Sparrow, A. H., *Can. Jour. Res., C*, **20**, 257–266 (1942).
[10] Matsuura, H., *Jour. Faculty Sci. Hokkaido Imp. Univ.* (Ser. 5), **3**, 233–250 (1935).
[11] Matsuura, H., *Cytologia*, **8**, 178–194 (1937).
[12] Huskins, C. L., and Wilson, G. B., *Ann. Bot.* (N. S.), **2**, 281–291 (1938).
[13] Wilson, G. B., and Hutcheson, I., *Can. Jour. Res., C*, **19**, 383–390 (1941).

TUMOR FORMATION BY ATTENUATED CROWN-GALL BACTERIA IN THE PRESENCE OF GROWTH-PROMOTING SUBSTANCES

BY ARMIN C. BRAUN AND THOMAS LASKARIS

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY, THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, N. J.

Communicated September 28, 1942

It has recently been shown[1, 2] that crown-gall tumor cells, when freed of the original inciting agent, *Phytomonas tumefaciens* (Smith and Town.), Bergey, *et al.*, are capable of autonomous development. The fact that these tumor cells when grown *in vitro* retain indefinitely their peculiar cultural and cytological characteristics as well as their tumor-inducing capacity in the absence of any demonstrable stimulating agent indicates that the host cells when acted upon by *P. tumefaciens* become permanently altered. They behave like malignant animal cells in that they (1) show uncontrolled growth in the initial host, (2) can be transplanted successfully to hosts of the same species from which they were derived or to closely related hosts and there continue their uncontrolled growth and (3) retain indefinitely their ability to produce tumors and to grow autonomously *in vivo*. The nature of the fundamental change which occurs in the cells is at present not known. However, a better understanding of the factor or combination of factors involved in tumor formation should be of aid in the clarification of the physiological basis for the development of this neoplastic growth.

In recent years a number of investigators have attempted to demonstrate a possible relationship between growth-stimulating substances and tumor development. Certain of these workers[3, 4, 5, 6] have drawn a parallel between the reaction of plant tissues treated with β-indole acetic acid, a substance produced by the crown-gall organism from tryptophane, and the reaction of similar plant material inoculated with a living culture of *Phytomonas tumefaciens*. Although Riker and his associates[7] have confirmed the experimental results of the above workers, they deny the importance of β-indole acetic acid or similar growth substances in tumor formation and conclude that, as far as they are aware, *P. tumefaciens* is pathogenic independently of these materials. Levine[8] agrees with Riker and states that no evidence has thus far been adduced to show that the mechanism of tumor formation is due to the presence of β-indole acetic acid.

The earlier work of Locke, Riker and Duggar[9] indicated, nevertheless, that there was a high level of growth-promoting substance present in plants inoculated with a virulent culture of the crown-gall organism. This was evidenced by marked growth responses such as epinasty of the leaf petioles, inhibition of the axillary buds, etc., that resulted, in addition

to tumor formation, in certain host plants. Plants inoculated with an attenuated culture show these growth responses to a much lesser degree or not at all. This would suggest that growth substances may be a limiting factor in tumor formation by the attenuated culture. Locke, *et al.*, were not able, however, to bring about an appreciable increase in the size of the tumors formed by supplementing the attenuated culture with β-indole acetic acid.

It is the purpose of the present paper to demonstrate that an attenuated culture of the crown-gall organism, when supplemented with certain growth-promoting substances, is capable of inducing the formation of large tumors in tomato plants. Evidence is presented, furthermore, that tissue fragments from such experimentally induced tumors retain undiminished their tumor-inducing capacity upon transplantation.

Methods.—The attenuated isolate of *Phytomonas tumefaciens* used in these experiments was first described by Hendrickson, *et al.*,[10] and designated by them as the *A*66 strain. This isolate is capable of inducing cellular proliferation at the point of inoculation somewhat in excess of the normal wound response of tomato plants, but it is unable to cause the development of crown-gall tumors under ordinary conditions (Fig. 1 (*b*)). Available for comparative purposes was the *A*6 strain from which the attenuated *A*66 culture was derived. The *A*6 strain is highly virulent, as shown by its ability to incite the formation of massive galls at points of inoculation (Fig. 1 (*a*)).

The host plant used throughout the tests was the tomato (*Lycopersicum esculentum* Mill. var. Bonny Best). Young seedlings were transplanted to 4-inch pots and kept on a greenhouse bench throughout the course of an experiment. When the plants reached a height of approximately 15 inches, they were decapitated and inoculated by means of the needle-puncture method with the attenuated *A*66 culture. Two inoculations were made in each plant at distances of approximately $^1/_4$ and $^3/_4$ inch below the decapitated stem. Four days after inoculation, chemically pure growth substances in concentrations of 0.5, 1.0, 1.5 and 2.0% in lanolin were applied separately to the decapitated stumps of the inoculated plants. At intervals of 7 days, the growth substances were renewed until 3 applications had been made. The growth substances used included α-naphthalene acetic acid, β-indole acetic acid and γ-indole butyric acid. Controls for the above experiments were set up as follows: One series of decapitated plants was punctured with a sterile needle instead of being inoculated with the attenuated culture but received the corresponding growth-substance applications; in a second series, pure lanolin was applied to plants inoculated with the attenuated culture.

The following technique, successfully employed by us in the tissue transplantation experiments to be described later, was adopted after a number

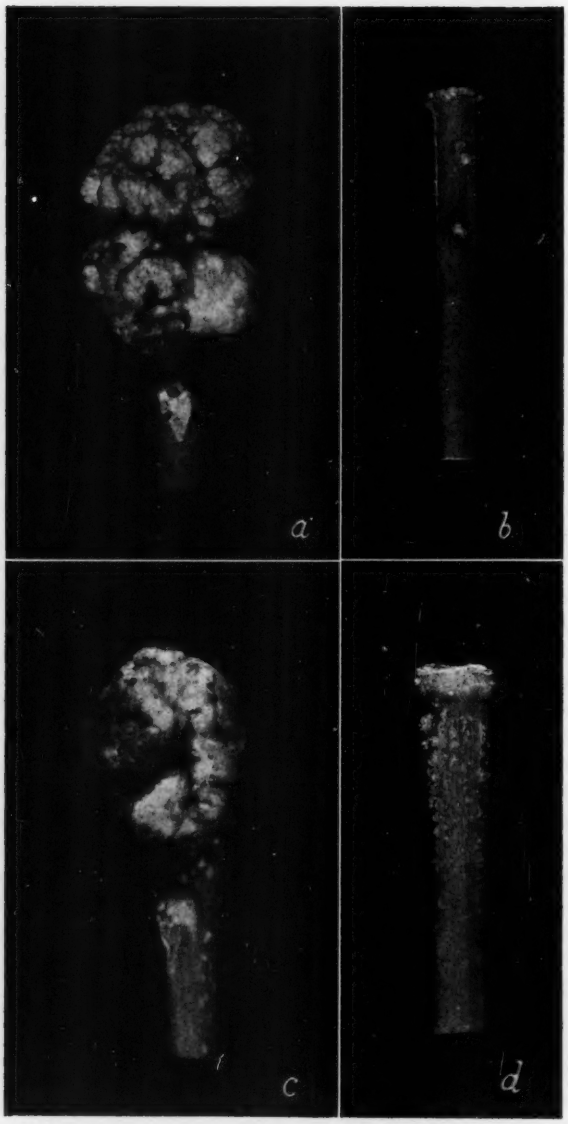

FIGURE 1

Decapitated tomato plants inoculated with (*a*) the virulent *A*6 culture, (*b*) the attenuated *A*66 culture, (*c*) the attenuated *A*66 culture and treated with synthetic growth-promoting substance. (*d*) Uninoculated, punctured with a sterile needle and treated with synthetic growth-promoting substance. Note similarity of tumors formed in (*a*) and (*c*). (Photographs by J. A. Carlile.)

of preliminary experiments established its superiority over the common grafting procedures. With the aid of a sharp scalpel, two parallel incisions were made along the axis of the stem, usually well above the middle of the plant. The incisions were approximately 1/4 inch apart and 1 inch in length. By careful insertion of the scalpel blade between the two incisions, the epidermal strip, together with the subjacent cortical and phloem elements, was pried loose from the main body of the stem and the desired tumor tissue fragment was then placed in the space between the flap and the stem. The implanted tissue was wrapped securely with "Sterilastic" tape which was removed 12 to 14 days later. The successful development of the implant appeared to be dependent upon early and satisfactory vascularization between the host and the tissue fragment. Tissues inserted inside or outside the confines of the vascular ring failed to develop in the majority of cases.

Experimental Results.—The three growth-promoting substances used, α-naphthalene acetic acid, γ-indole butyric acid and β-indole acetic acid were all found to have a pronounced stimulating effect causing the development of large tumors in plants that had been previously inoculated with the attenuated *A*66 culture. However, consistent differences in the degree of stimulation, rate of tumor development and morphology of the resulting tumor mass were exhibited by the growth-promoting substances. The stimulating action of γ-indole butyric acid was usually observed somewhat earlier than that of α-naphthalene acetic acid. The latter, however, gave rise to tumors that most closely resembled those initiated and stimulated by the virulent *A*6 culture. These tumors were white in color and irregular in contour, while those produced with the aid of γ-indole butyric acid were regular in contour and had a decidedly greenish tinge. In most instances the tumors stimulated by these two substances grew rapidly and became very large. Sizable tumors frequently developed after 3 applications of 0.5% of these growth substances in lanolin or following a single application of 1.0%. β-Indole acetic acid was not as effective in our hands as were the other two materials. Higher concentrations, usually 3 applications of 2.0% in lanolin, were required to bring about a tumor stimulation comparable to that secured with lower concentrations of the other two substances. The β-indole acetic acid-stimulated tumors, however, frequently grew to be very large and resembled closely those stimulated by γ-indole butyric acid. Figures 1 (*c*), 2 (*a*) and 2 (*b*) show, respectively, the stimulatory effects of 3 applications of 1.0% of α-naphthalene acetic acid, 2.0% of β-indole acetic acid and 1.0% of γ-indole butyric acid in lanolin. The control, an attenuated culture-inoculated plant treated with pure lanolin, is shown in figure 1 (*d*).

The stimulatory effects of these growth substances on tumor development by the attenuated culture were not always evident even though

conditions were favorable. Thus, under completely comparable conditions, tumors on some plants would be stimulated to further development while not on others. The explanation of this variability may involve differences in rate of diffusion of growth-promoting substances in different plants.

The action of α-naphthalene acetic acid, β-indole acetic acid and γ-indole butyric acid on tomato plants has been studied by many investigators in recent years. In our work as well as in the work of others it has been found that these substances in the concentrations commonly used are

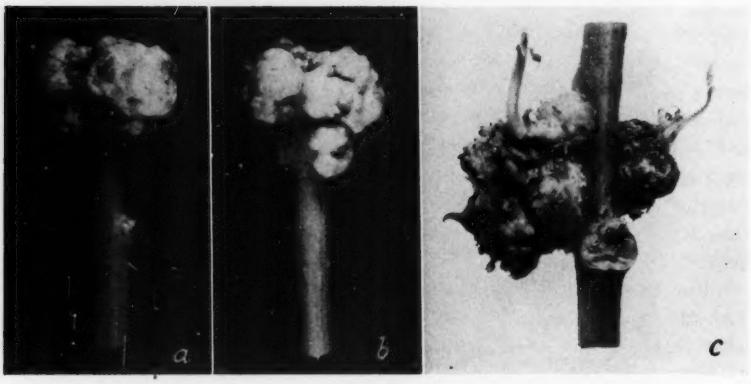

FIGURE 2

Decapitated tomato plants inoculated with the attenuated *A*66 culture and stimulated with (*a*) β-indole acetic acid, (*b*) γ-indole butyric acid and (*c*) the plant growth hormones. (Photographs by J. A. Carlile.)

essentially root stimulants, although some hypertrophied and hyperplastic tissues are formed. The latter present a histological picture not unlike that of crown-gall tumor tissue. The hypertrophied and hyperplastic cells are, however, endowed with very limited powers of proliferation and continue to develop only as long as they are kept stimulated by the growth substances. We have never observed overgrowths to be produced on tomato plants even by repeated applications of these substances that are comparable either in size or rate of development to those produced by the crown-gall organism. Figure 1 (*a*) shows the typical reaction of a decapitated tomato stem which had received 3 applications of 1.0% of γ-indole butyric acid in lanolin. A limited amount of cellular proliferation has occurred at the cut surface where the growth substance was applied; below the cut surface there are found numerous root primordia which under humid conditions would develop into true roots. The action of

α-naphthalene acetic acid and β-indole acetic acid on decapitated tomato stems is very similar.

The discovery that synthetic growth substances were able to stimulate the development of tumors by the attenuated culture strengthened our previous belief regarding the probable rôle of the host growth hormones in the development of these neoplastic growths. It had been observed that the removal of axillary shoots from decapitated tomato plants inoculated with the attenuated *A*66 culture frequently resulted in forcing the development of small adventitious buds on or in the proximity of the proliferating tissue. The further expansion and development of these buds were often accompanied by an expansion in the mass of the subjacent tumor tissue which increased at a tremendous rate and finally reached extremely large dimensions (Fig. 2 (*c*)). Because expanding buds are known to be a rich source of growth substances, it appears likely that the plant hormones secreted by the buds served as the stimulating agent in much the same manner as did the synthetic growth-promoting materials described above. No such stimulation was observed in those cases where the adventitious buds failed to develop. There is little question but that these "leafy galls" are composed largely of tumor tissue. Our evidence for this claim will be advanced in a later section.

The possibility that the application of the growth substances had significantly altered the degree of virulence of the attenuated culture was also investigated. Bacterial isolations were made from many of the artificially stimulated tumors by means of the usual poured-plate procedures. Large numbers of the bacterial colonies that developed were reinoculated into healthy tomato plants. In addition, tumor tissue fragments were thoroughly ground up with a small amount of nutrient dextrose broth in a mortar and the resulting suspension inoculated directly into healthy tomato plants. The results from many such tests demonstrated conclusively that the attenuated culture had not increased in virulence. It still produced only slight proliferation comparable to that produced by the stock *A*66 culture. The conclusion seems justified, therefore, that the primary effect of the growth substances was to stimulate the host cells rather than to bring about a change in the virulence of the bacteria themselves. Additional evidence in support of this conclusion is presented below.

Transplantability of Tumor Tissue.—Perhaps the most distinctive characteristic of malignant cells is their relative independence of the conditions which govern the growth of normal tissues. All normal cells are subject to a rigid control mechanism when growing *in vivo*, and it is only transformed cells such as are found in neoplasms that react differently. These abnormal cells fail to respond to the morphogenetic restraints which direct the growth of cells in a healthy organism. As was pointed out earlier,

crown-gall tumor cells, like most malignant animal cells, appear to be of a type which is capable of unlimited transplantability and tumor-inducing capacity under favorable conditions. We have used, therefore, these criteria in determining whether the attenuated *A*66 culture of *Phytomonas tumefaciens* is capable, as is the virulent culture, of permanently altering the host cells.

Fragments from tumors initiated by the attentuated culture and stimulated with α-naphthalene acetic acid, γ-indole butyric acid and the natural plant growth hormones were implanted under the epidermis of tomato plants. It was soon observed that in those cases where a vascular connection with the host was clearly established the tissue fragments developed into typical crown-gall tumors in relatively short periods of time. This was especially true of the α-naphthalene acetic acid and γ-indole butyric acid-stimulated tissue which in many instances developed into large tumors 3 to 4 cm. in diameter within a period of 4 to 5 weeks. On the other hand, the tissue fragments from the plant growth hormone-stimulated "leafy" galls developed more slowly and usually did not exceed 1.5 to 2 cm. diameter in 5 weeks of development.

As indicated above, the gross morphological characteristics of the tumors initiated by the attenuated culture and stimulated by various growth substances differed considerably. However, upon transplantation of the various tumors, all of the resulting overgrowths appeared similar and could not be distinguished from each other except possibly by their respective rates of development. The tumors were for the most part a very light brown in color and of a fairly regular contour.

We have now successfully carried some of these tumors through 5 successive passages in tomato plants over a period of more than 6 months. These tumors have continued to develop at an undiminished rate throughout this period. Figure 3 (*a*) shows a typical third-generation tumor transplant after 4 weeks of development.

Additional bacterial isolations were made from these transplanted tumors to determine whether the degree of virulence of the attenuated culture had been in any way altered during the course of the experiments. It was found after testing several hundred isolates on tomato plants that no increase in virulence over that of the stock *A*66 culture had occurred. It seemed logical to assume, therefore, that the mere presence of these attenuated organisms in the tissues could not account for the rapid expansion of the transplanted fragments and that it must be the host cells themselves that had been altered to such a degree that they were now developing autonomously. This assumption was definitely established by our subsequent discovery that certain tissue fragments of the γ-indole butyric acid-stimulated tumors were entirely free of *Phytomonas tumefaciens*. Whether the bacteria had died out in these fragments or whether

the rapidly developing tumor tissue had grown away from the bacteria is not known. The bacteria-free tissues have been carried through 3 transplant generations involving a period of more than 3 months. All attempts to demonstrate the presence of the crown-gall bacteria in these

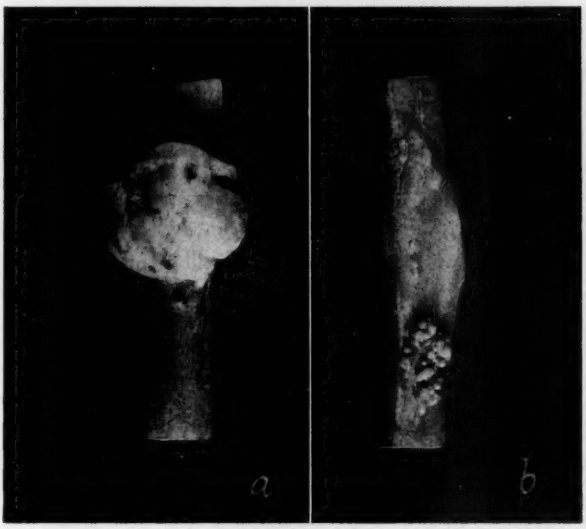

FIGURE 3

(*a*) Typical third-generation implant (originally derived from an experimentally induced tumor, Fig. 1 (*c*)) after 4 weeks of development. (*b*) Typical first-generation implants of tissue stimulated with growth-promoting substance (see Fig. 1 (*d*)) after 6 weeks of development. Two general types of response are illustrated. In the upper implant the tissue remained smooth and made but slight growth. The nodular root-like protuberances and limited growth of the lower implant characterized the second type of response. (Photographs by J. A. Carlile.)

tissues by the usual isolation procedures have failed. The bacteria-free tumor tissue fragments grew rapidly and developed into large neoplastic growths indistinguishable morphologically from the bacteria-containing tumors. On the basis of these results, it can be stated, therefore, that the attenuated bacteria are capable of altering the host cells to tumor cells which after stimulation are apparently capable of autonomous development.

The controls for the experiments described in the preceding pages were uninoculated decapitated tomato plants treated with 1.0, 1.5 and 2.0%

of β-indole acetic acid and α-naphthalene acetic acid in lanolin, as explained earlier. These materials were found to be essentially root-forming substances, although they did cause a limited amount of cellular proliferation. Fragments of these growth substance-stimulated tissues were grafted into tomato plants in the same manner as were the tumor tissue fragments. Similar grafting experiments were carried out on three separate occasions with the object of determining whether the growth substances used here were capable of permanently altering the host cells. In no instance did we find evidence suggesting that such a change had occurred. The tissue implants seldom increased to more than twice their original size and never developed into tumors. Two general types of responses were observed (Fig. 3 (*b*)). In one type of response the tissue implant gave rise to numerous nodular root-like protuberances which seldom developed into true roots, although occasionally roots 1 to 2 cm. in length were observed. The second type could be likened to a slow healing response, the inserted tissue remaining smooth and making but slight growth. It is apparent that the host cells, when acted upon by these growth substances alone, are incapable even after successful transplantation of inducing the formation of tumors. It is our belief that the growth substances used in this study served merely to stimulate cells previously altered by the attenuated culture.

Discussion and Conclusions.—It has been demonstrated that an attenuated culture of *Phytomonas tumefaciens* is capable of forming large tumors in tomato plants when supplemented with the plant growth hormones or synthetic growth-promoting substances. This growth substance reaction does not appear to be specific inasmuch as any one of several different growth substances, including that produced by the host plant, may serve as the stimulating agent. Furthermore, the gross morphological appearance of the tumors formed when an attenuated culture is supplemented with a growth substance varies with the substance used. Of such tumors, those stimulated by α-naphthalene acetic acid approximate most closely those induced by the virulent culture on tomato in that they are white in color and irregular in contour.

It has further been demonstrated by means of grafting experiments that the cells of these experimentally induced tumors can be transplanted in series and subsequently develop into large tumors which may reach a size of 3 to 4 cm. in a period of 5 weeks. Since certain of these tumors are apparently bacteria-free and since it has been shown in the case of those tumors which are not bacteria-free that the degree of virulence of the attenuated culture itself has not been altered by the application of growth substances, it is concluded that the attenuated culture like the virulent culture is capable of altering the host cells to tumor cells. However, the attenuated culture alone is unable to stimulate the altered cells to any

appreciable extent. When artificially stimulated with growth substances, these altered cells are apparently capable, as are those stimulated by the virulent culture, of uncontrolled growth *in vivo*. Thus, there appear to be at least two distinct phases involved in tumor formation. In the first phase, the normal host cells are changed to tumor cells which without stimulation will not develop into a neoplastic growth. The second phase consists in the stimulation of the changed cells to continued multiplication by a growth substance, resulting ultimately in the formation of a tumor.

The question as to whether a single substance or two or more substances are involved in the alteration and stimulation of the tumor cells remains as yet unanswered. If only a single substance is involved, however, it must differ from the growth substances used here because the cells of the tomato plant stimulated by these substances alone do not, upon transplantation, induce the formation of tumors. If two or more substances are involved, then one of the common growth-promoting substances might conceivably act as the stimulating agent. The difference between the virulent and the attenuated cultures may lie in the relative amounts of growth substances produced by each, either as a product of bacterial metabolism or by the host under the influence of these organisms. The fact that pronounced growth substance responses are characteristically shown by tomato plants inoculated with the virulent culture and are absent in similar plants inoculated with the attenuated culture lends credence to this belief.

Once the altered host cells are sufficiently stimulated, they are apparently capable of indefinite multiplication and tumor formation under favorable conditions without the additional application of growth-promoting substances. The altered cells then continue to multiply autonomously and retain, perhaps indefinitely, their tumor-inducing capacity.

[1] Braun, A. C., and White, P. R., *Phytopath.* (in press).
[2] White, P. R., and Braun, A. C., *Cancer Res.*, **2**, 597–617 (1942).
[3] Berthelot, A., and Amoureux, G., *C. R. Acad. Sci. Paris*, **206**, 537–540 (1938).
[4] Brown, N. A., and Gardner, F. E., *Phytopath.*, **26**, 708–713 (1936).
[5] Kraus, E. J., Brown, N. A., and Hamner, K. C., *Bot. Gaz.*, **98**, 370–420 (1936).
[6] Link, G. K. K., Wilcox, H. W., and Link, A. DeS., *Ibid.*, **98**, 816–867 (1937).
[7] Riker, A. J., Henry, B., and Duggar, B. M., *Jour. Agr. Res.*, **63**, 395–405 (1941).
[8] Levine, M., *Bull. Torrey Bot. Club*, **67**, 199–226 (1940).
[9] Locke, S. B., Riker, A. J., and Duggar, B. M., *Jour. Agr. Res.*, **57**, 21–39 (1938).
[10] Hendrickson, A. A., Baldwin, I. L., and Riker, A. J., *Jour. Bact.*, **28**, 597–618 (1934).

THE RÔLE OF INTRACELLULAR CATIONS ON LIVER GLYCOGEN FORMATION IN VITRO

BY A. BAIRD HASTINGS AND JOHN M. BUCHANAN

DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL

Communicated October 14, 1942

The importance of specific inorganic ion concentrations in the extracellular fluids of the body for the maintenance of the integrity and normal function of cells has long been recognized. The development of "balanced" salt solutions, such as Ringer's solution, as a substitute for natural extracellular fluid is an illustration of this. In recent years, attention has turned, however, to the importance of the intracellular ion concentrations for the maintenance of normal intracellular enzyme activity.[1,2] This becomes of special urgency in *in vitro* studies in which metabolic functions of tissues are under investigation. For example, the intracellular fluid of mammalian liver is high in potassium and magnesium and low in (or perhaps free from) sodium and calcium; whereas the extracellular fluid of the tissue is similar to blood plasma, high in sodium and low in potassium, magnesium and calcium. When liver tissue is incubated *in vitro* at body temperature in an artificial extracellular medium such as Ringer's solution, there is a progressive change in permeability of the cells with a resultant exchange of intracellular and extracellular ions until equilibrium is established. Such an exchange of ions results in an intracellular ionic environment which is quite different from that which normally obtains in the body.

However, by placing tissues in a fluid similar to the intracellular fluid with respect to its cations, it should be possible to maintain the normal intracellular cation environment in spite of exchange of ions across the cell boundary.

The present communication reports the effects the differences in ionic environment produced upon one metabolic function of rat liver—namely, the production of glycogen from glucose *in vitro*. Our experiments were stimulated by the work of Ostern, Herbert and Holmes[3] who reported in 1939 the *in vitro* production of glycogen by rabbit liver when incubated with glucose in Ringer's solution enriched with calcium. These experiments with rabbit liver were repeated and confirmed in our laboratory, but rat liver, under the same conditions, failed to produce significant amounts of glycogen. We, therefore, turned to a more critical examination of the effect of intracellular versus extracellular cations on glycogen formation.

The specific problem undertaken was the comparison of the glycogen content of liver slices before and after they had been incubated for one hour at 38°, in isotonic glucose-containing solutions: (*a*) similar to intracel-

lular fluids with respect to cations—i.e., high in potassium and magnesium, and (*b*) similar to extracellular fluids—i.e., high in sodium with small amounts of potassium, calcium and magnesium. Comparable experiments were also performed in the absence of glucose. These provided information on the rôle of cations on net glycogen formation and breakdown. In view of the complexity of the intracellular organic phosphate anions, it is impractical at present to provide a normal intracellular anionic environment in the extracellular medium.

It may be stated at once that incubation of the liver in the high potassium, high magnesium medium favored glycogen synthesis, whereas incubation in the high sodium medium favored glycogen breakdown.

Experimental.

SOLUTION	Na, mM/L	K, mM/L	Ca mM/L	Mg, mM/L	Cl, mM/L	HCO_3, mM/L	GLUCOSE %
I	0	130	0	20	130	40	1.0
II	152	5	1	1	121	40	1.0
III	0	130	0	20	130	40	0
IV	152	5	1	1	121	40	0

The above solutions were equilibrated with 5% CO_2:95% O_2. When 2 cc. of such solutions were incubated in 25 cc. vessels with 300–500 mg. of rat liver slices for one hour, the resultant pH was found to be between 7.35 and 7.45. The glycogen content of liver plus the solution of each vessel was determined by the method of Good, Kramer and Somogyi[4] as modified by Sjörgen.[5] The total phosphorus content of each vessel was also determined by the method of Fiske and Subbarow.[6] The initial glycogen and phosphorus contents of the liver were determined before incubation in order to provide a basis for comparison. It was found to be more convenient to estimate the amount of liver in each vessel from the phosphorus determinations than to depend upon the weights of the slices of liver which was introduced. Results were calculated in terms of grams of glycogen per 100 grams of wet liver. The rats were starved for 18 hours prior to experimentation.

Results.—In 37 out of 42 experiments in which rat liver, whose initial glycogen was less than 0.3 per cent, was incubated with Solution I (i.e., high K and Mg), there was an absolute increase (average 0.05–0.15%) in the amount of glycogen present at the end of one hour. In experiments in which the initial glycogen was greater than 0.3 per cent, a decrease in glycogen was usually observed. It was concluded from these experiments that, contrary to the previous experience in which little or no increase in glycogen occurred when Ringer's solution was used, rat liver would quite consistently produce glycogen *in vitro* when incubated in a potassium-mag-

nesium medium providing the initial glycogen concentration did not exceed 0.3 per cent.

The results obtained in seven experiments in which the effects of using Solutions I, II, III, IV were compared are shown graphically in figures 1

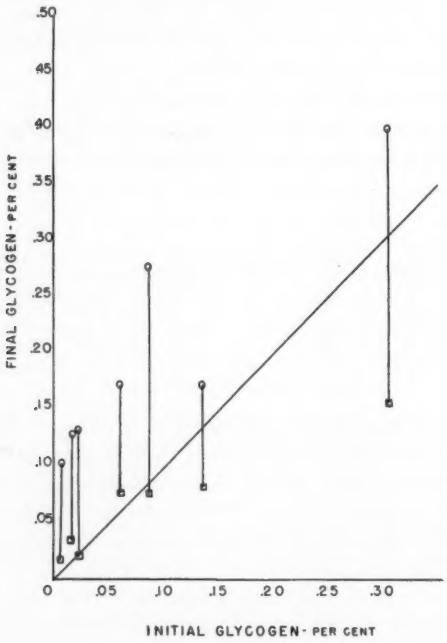

FIGURE 1

Final liver glycogen concentration is plotted against initial glycogen concentration. Circles designate experiments with Solution I (high potassium and magnesium, containing glucose); squares designate experiments with Solution II (high sodium containing glucose). Points lying below the diagonal line indicate net glycogen breakdown, points above the line indicate net glycogen formation.

and 2. In figure 1, it will be seen that, in the presence of glucose, the final glycogen concentration was absolutely greater than the initial concentration when the high potassium-magnesium medium (I) was used; whereas, when the high sodium medium (II) was used, the final glycogen concentration was less than or essentially the same as the initial concentration. Figure 2 shows that, in the absence of glucose, there was no loss of glycogen

in the high potassium-magnesium medium (III), whereas, the glycogen practically disappeared in the high sodium medium (IV). These results may be interpreted as indicating that high sodium and low potassium, cal-

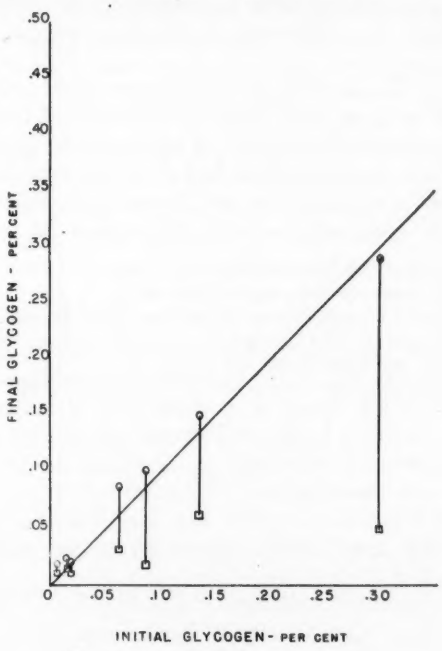

FIGURE 2

Final liver glycogen concentration is plotted against initial glycogen concentration. Circles designate experiments with Solution III (high potassium and magnesium, without glucose); squares designate experiments with Solution IV (high sodium, without glucose). Points lying below the diagonal line indicate net glycogen breakdown, points above the line indicate net glycogen formation.

cium and magnesium favored glycogen breakdown in excess of glycogen synthesis, whereas, the high potassium and high magnesium solution favored glycogen synthesis in excess of glycogen breakdown.

The above observations are to be regarded as only preliminary to a more detailed and complete analysis of the role of ions in influencing intracellular reactions. For example, it has already been observed in this laboratory by one of us (J. M. B.) that calcium is more effective than magne-

sium in promoting glycogen synthesis in rat liver *in vitro*. It is not the intention of the present communication to consider the question of what solution would provide for optimum rate of glycogen formation, but rather to call attention to the importance of considering the maintenance of the normal intracellular ionic environment in the study of intracellular enzymic reactions.

Summary.—1. The presence of concentrations of potassium and magnesium comparable to those occurring in intracellular fluid has been found to favor glycogen formation by rat liver *in vitro*, whereas incubation of liver in media comparable to extracellular fluid favors glycogen breakdown.

2. The importance of maintaining a normal intracellular ionic environment in the study of intracellular reactions is pointed out.

[1] Fenn, W. O., *Physiol. Rev.*, **20**, 377 (1940).
[2] Hastings, A. B., *Harvey Lectures*, **36**, 91 (1940–1941).
[3] Ostern, P., Herbert, D., and Holmes, E., *Biochem. Jour.*, **33**, 1858 (1939).
[4] Good, C. A., Kramer, H., and Somogyi, M., *Jour. Biol. Chem.*, **100**, 485 (1933).
[5] Sjögren, B., *Arch. ges. Physiol.*, **250**, 427 (1938).
[6] Fiske, C. H., and Subbarow, Y., *Jour. Biol. Chem.*, **66**, 375 (1925).

ON THE MATERIAL EJECTED FROM NOVAE

BY CECILIA PAYNE-GAPOSCHKIN AND SERGEI GAPOSCHKIN

HARVARD COLLEGE OBSERVATORY

Communicated October 16, 1942

The extremely complex spectroscopic problems connected with the outbursts of novae present many angles of approach. The one that has been most thoroughly exploited is concerned with the identification of the several absorption and emission spectra, moving with different radial velocities through the ejected envelope. The problem cannot, however, be regarded as solved until not only the motion and distribution, but also the quantity and composition of the ejected matter has been analyzed. The present paper is concerned with an approach to the second of these questions.

In studying the changes in the spectra of novae during and after the outbursts it has been usual to estimate and describe the intensities of the dark and bright lines as seen against the continuous background of the spectrum. Such estimates, however, are deceptive, since the intensity of the background is itself changing rapidly (and not necessarily uniformly), so that the true changes, especially in the intensities of bright lines, may be falsified.

Such lines are often described as increasing in intensity with time, but when true intensities are measured, it is found that increases of intensity for bright lines in the spectra of novae are very uncommon.

1. The Material.—The intensities in the bright lines of novae have been under investigation for a number of years, principally on the basis of the Harvard collection of objective prism spectra.[1] The problems concerned with the use of photometrically unstandardized spectra, and the methods used for the determination of intensities, are sufficiently described in the papers cited. The intensities of the more prominent bright lines in the spectra of five bright novae (Nova Persei 1901, Nova Aquilae 1918, Nova Cygni 1920, Nova Pictoris 1925 and Nova Herculis 1934) are given for several months after the first outbursts. The object of the present note is to summarize the results of these investigations, and to draw some elementary conclusions about the quantity and condition of the material ejected.

2. Summary of Results.—The lines of hydrogen, helium (neutral and ionized), ionized carbon, twice ionized nitrogen and ionized iron are the principal permitted radiations shown by novae, and measures of all of these have been made for at least some of the stars. Furthermore, the "forbidden lines" of once ionized nitrogen, of neutral and twice ionized oxygen, of once ionized sulphur and of twice ionized neon, have been measured. It must be remembered that the bright lines of novae, like the absorption lines, are of complex structure, and that the measures refer to the whole line (undoubtedly formed at a variety of spots in an inhomogeneous, expanding envelope); quantitative results drawn from them are therefore only approximate, and refer essentially to conditions averaged over the volume from which the given lines emanate.

The measured intensities of the bright lines (which are fully tabulated, day by day, in the papers cited) are determined in ergs per square centimeter per second at the surface of the earth. The greatest intensities recorded are about 10^{-6} ergs per second per square centimeter; the smallest intensities attainable with our material are about 10^{-10} ergs per second per square centimeter. These quantities have little significance in comparing the data for different novae, which are at very different distances; a physical discussion requires a knowledge of the energy emitted per second *at the nova*. The necessary factors are derived in table 1.

TABLE 1

REDUCTION TO ABSOLUTE INTENSITY

NOVA	ABSOLUTE VISUAL MAGNITUDE	MODULUS	LOGARITHM OF REDUCTION FACTOR
Per 1901	− 8.7	8.7	43.56
Aql 1918	−10.0	8.6	43.52
Cyg 1920	− 8.7	10.7	44.34
Pic 1925	− 7.7	8.9	43.64
Her 1934	− 7.1	8.5	43.48

For comparison, the absolute intensities radiated in the hydrogen line Hδ by the five different novae at a number of different absolute magnitudes are shown in table 2. The intensity passes through a flat maximum, and thereafter falls off as the star fades, but not as rapidly (so that, relative to the continuum, it appears to strengthen).

TABLE 2

ABSOLUTE INTENSITY OF Hδ (LOGARITHMS OF ERGS PER SECOND)

ABSOLUTE MAGNITUDE	NOVA PERSEI	NOVA AQUILAE	NOVA CYGNI	NOVA PICTORIS	NOVA HERCULIS
−10	...	36.58	...	...	...
− 9	...	36.58	...	...	...
− 8	36.62	36.92	36.95	...	...
− 7	36.52	36.94	36.87	35.72	35.24
− 6	36.21	36.60	36.62	36.04	35.32
− 5	35.80	36.42	36.25	35.44	34.68
− 4	35.44	36.14	35.84	35.46	33.36
− 3	35.14	35.80	35.54	35.04	34.08
− 2	34.85	34.82:	35.30	34.80	34.11
− 1	...	...	35.00	34.36	...

The intensity of the sum of the nebular lines of [OIII], N_1 and N_2 is similarly summarized in table 3. Here the intensity remains rather steady, though from mere inspection of the spectra it appears to increase rather strongly.

TABLE 3

ABSOLUTE INTENSITY OF THE NEBULAR LINES

ABSOLUTE MAGNITUDE	NOVA PERSEI	NOVA AQUILAE	NOVA CYGNI	NOVA PICTORIS	NOVA HERCULIS
−5	...	37.10	...	...	...
−4	...	37.28	...	...	34.48
−3	35.58	37.28	35.85	35.34	35.32
−2	35.18	37.28	35.82	35.34	35.90

3. Physical Conditions. The Excitation Temperatures.—The intensities of the bright lines furnish several clues to the physical condition of the material ejected. Some approximation to the problem may be obtained by treating the ejected envelope as though it were the envelope of a planetary nebula, with which a nova presents obvious analogies. The intensities of the bright lines, when compared to the corresponding intensities in the envelopes of planetaries, lead to temperatures for the exciting nuclei of novae that are very comparable to the temperatures in the central stars of the planetary nebulae. The envelope of N.G.C. 7027, for instance, can be compared with those of the novae by comparing our measured relative intensities with those published by Wyse[2] for that object. The excitation temperature for Nova Pictoris is found to be rather higher than for the

nebula, that of Nova Herculis, about the same and those of the other three novae, a little lower. It may be remarked that Nova Pictoris showed the highest excitation lines ever recorded for a nova, in the great intensity of [Fe VII][3] in the 1931 spectrum, as described by Spencer Jones;[4] a high temperature is, therefore, not surprising. The temperature of the central star of N.G.C. 7027 is estimated by Aller[5] as 88,000°. By another method, Sayer[6] deduced that the excitation temperature of the nuclear star of Nova Ophiuchi 1933 (RS Ophiuchi) could not have been less than 10^5 degrees.

4. Physical Conditions. The Electron Temperatures.—The electron temperatures in the envelopes of the novae may be deduced, by the methods developed by Menzel, Aller and Hebb[7] for the envelopes of planetary nebulae, from the ratio of intensity of the auroral to the nebular lines of [OIII]. If the electron density is high enough, a similar method may be used for [OI]. The resulting electron temperatures are summarized in table 4.

TABLE 4

ELECTRON TEMPERATURES FOR FIVE NOVAE

ABSOLUTE MAGNITUDE	NOVA PERSEI [OIII]	NOVA AQUILAE [OIII]	NOVA CYGNI [OIII]	NOVA PICTORIS [OIII]	NOVA HERCULIS [OIII]	NOVA HERCULIS [OI]
−7	..	..	..	..	..	6600
−6	..	..	..	..	..	6500
−5	..	5900	..	..	..	3900
−4	..	5500	..	..	7300	3500
−3	8100	5800	5800	8400	7200	..
−2	7300	5500	5900	6700	6100	..
−1	..	..	5600	6300	..	..

The electron temperatures are low, and this is readily accounted for by the depressing effect[8] of the abundance of OIII, and also, for several of the novae, Ne III. The very low electron temperatures deduced for Nova Herculis from the [OI] lines are without doubt due to the departure from the assumed Boltzmann distribution for this atom.

5. Physical Conditions during the Secondary Fluctuations.—Many novae have undergone fluctuations on the decline from maximum, the most striking cases being those of Nova Persei and Nova Aquilae. It has been shown elsewhere for Nova Aquilae[9] that the fluctuations are even more marked in the nuclear star than in integrated light, and these fluctuations have been interpreted[10] as oscillations of temperature, accompanied by apparent contractions and expansions of the central star as the temperature rises or falls. This phenomenon is in harmony with the conclusions drawn[11] for Nova Herculis on the hypothesis of continuous ejection: "The position of the photosphere. . .is governed, not by the distance to which the ejected matter has traveled, but by the physical state of the extended atmosphere and the amount of energy incident on it from beneath. The photosphere

is stationary...but matter passes through it continuously from below..." The drops in brightness, then, should represent rises of temperature and apparent contractions of the central star.

Spectroscopic evidence that this is the case is shown in figure 1, which follows the change in the ratio of He I to He II lines through typical minima in the secondary fluctuations of Nova Persei and Nova Aquilae. The ratio falls sharply during the minima, which may be ascribed to increased ionization and associated with a rise in temperature.

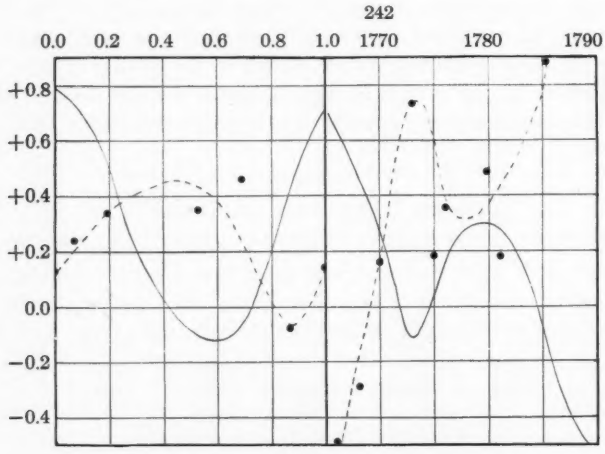

FIGURE 1

Ionization of helium during the great fluctuations of Nova Persei (left) and Nova Aquilae, Abscissae are fractions of the period of oscillation for Nova Persei, Julian days for Nova Aquilae. Ordinates are logarithms of the ratio of intensity of 4471 He I to 4686 He II. Continuous lines represent the course of the light curve, broken lines, that of the He I/He II ratio. The diagram for Nova Persei is composite; that for Nova Aquilae represents continuous observations.

6. The Electron Densities.—The analogy with the envelopes of planetary nebulae may be used to estimate the electron densities in the expanding atmospheres of novae. The method given by Menzel and Aller[12] was used, with the modifications necessary to apply to Hδ (the line given in our tables), and revised values of the constants, kindly supplied by Dr. Aller.

The electron densities, which are illustrated for a number of absolute visual magnitudes for the five novae, in figure 2, are from 10^8 to 10^9 per cubic centimeter at the beginning of the outburst, and fall to between 10^5 and 10^6 per cubic centimeter during the interval covered by our measures. This

value is interesting as falling between those given by Menzel and Aller[13] for the envelopes of planetary nebulae (10^3 to 10^4 per cubic centimeter) and those calculated by Aller[5] for the envelopes of Wolf-Rayet stars (from 10^{12} to 10^{13} per cubic centimeter).

7. *The Ejected Masses.*—On the very simple assumption that every electron is matched in the atmosphere by a hydrogen nucleus, the data of figure 2 may be used to estimate the masses ejected during the first few

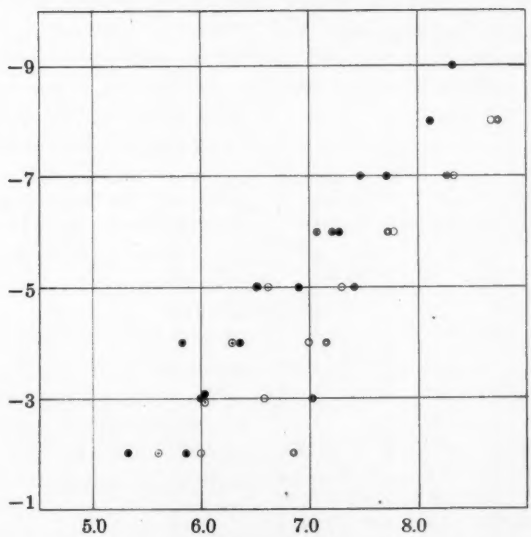

FIGURE 2

Electron densities and absolute magnitudes for five novae. Abscissae are logarithms of electron densities per cubic centimeter; ordinates are absolute visual magnitudes. The novae are distinguished as follows: Nova Persei, circles; Nova Aquilae, dots; Nova Pictoris, small dots in circles; Nova Cygni, two circles; Nova Herculis, large dots in circles.

months. The calculated masses are shown in figure 3. Evidently they continue to increase long after the time at which we must suppose ejection (in any considerable quantities) to have ceased. That this increase is real seems unlikely. More probably the hydrogen was largely ionized at the maximum, and later recombined; thus the inferred number of hydrogen atoms per cubic centimeter in the early stages (perhaps in all stages) would be too small. The assumption that equal numbers of hydrogen nuclei and electrons are present can, however, be replaced by a better one, as will be

shown in a later note. The modification alters our view concerning the number of atoms of hydrogen, but the order of the deduced masses is not changed.

It seems likely that the values deduced at the end of the period studied are nearest to the actually ejected masses. Nova Aquilae stands out as having ejected the greatest mass, just as it stands out in luminosity. Luminosity and ejected mass are compared in figure 4. The three high-weight novae (for which the actual rates of expansion of the ejected neb-

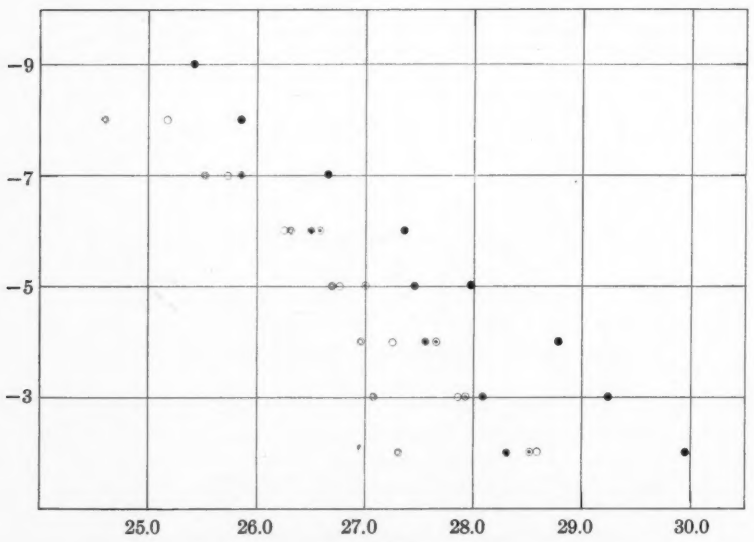

FIGURE 3

Ejected masses for five novae. Abscissae are logarithms of ejected masses, in grams. Ordinates are absolute visual magnitudes. The novae are denoted by symbols as in figure 2.

ulae are known) are shown by larger symbols in the figure. For Nova Cygni and Nova Pictoris, the size of the hypothetical expanding envelope was calculated from the radial velocities, and an arbitrary choice of velocity had to be made. A larger velocity than the 750 km./sec. used for Nova Cygni would have reduced the electron densities in the later stages, increased the deduced masses and brought it into accordance with the other novae in figure 4.

The straight line drawn in figure 4 represents proportionality between ejected material and luminosity at maximum. It is given by the relation

$$\log \text{mass} = -0.4M_{vis} + 25.5,$$

where the mass is given in grams. The three high-weight novae do not deviate much from this line.

Other estimates of the masses ejected from novae are in general agreement with the foregoing. Dr. Whipple and one of the writers[14] found that Nova Herculis ejected a mass of the order of 10^{29} grams up to the time of the great minimum. Sayer[6] calculated that 10^{28} grams were ejected by Nova Ophiuchi 1933. A similar estimate, 10^{-5} to 10^{-6} solar masses, is given[15] by Ambarzumian and Gordeladze. For Nova Lacertae 1936, Popper[16] has derived masses of 6×10^{-5} and 7×10^{-5} solar masses, from electron densities of the order of 10^{6} per cubic centimeter about 100 days after maximum light.

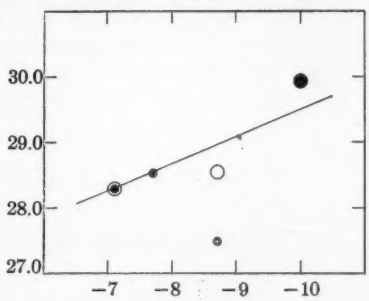

FIGURE 4

Relation of ejected mass to luminosity at maximum. Ordinates are logarithms of ejected masses in grams. Abscissae are absolute visual magnitudes at maximum. The five novae are denoted by the same symbols as in figures 2 and 3. Points of lower weight are shown by small symbols. The straight line represents the relation: log mass $= -0.4M_{vis} + 25.5$.

It is hoped to study the composition of the ejected material in a later communication.

8. Summary.—The present note draws some elementary physical conclusions from the current measures of the intensities of the bright lines in the spectra of five novae. The excitation temperatures are very high—comparable to those in the nuclei of planetary nebulae. The electron temperatures are less than 10,000°, not very different from those in the envelopes of planetary nebulae. The great fluctuations of Nova Persei and Nova Aquilae were occasioned by sharp rises and falls of excitation temperature.

The electron densities, estimated by analogy with the envelopes of planetary nebulae, are from 10^{8} to 10^{9} per cubic centimeter at the outburst, and fall (during the interval studied) to 10^{5} or 10^{6} per cubic centimeter. Thus the expanding envelopes of novae are intermediate between the planetary nebulae and the envelopes of Wolf-Rayet stars.

The mass of ejected material is estimated at from 10^{27} to 10^{30} grams for the five novae studied. These masses appear to be in rough proportion to the luminosities of the corresponding novae at maximum.

[1] Whipple, F. L., and Payne-Gaposchkin, C., *Harv. Obs. Circ.*, No. 414, 1–13 (1937); Payne-Gaposchkin, C., and Whipple, F. L., *Ibid.*, No. 433, 1–12 (1939); Payne-

Gaposchkin, C., and Menzel, D. H., *Ibid.*, No. 428, 1–13 (1936); Payne-Gaposchkin, C., and Gaposchkin, S., *Ibid.*, No. 445, 1–19 (1942).

[2] Wyse, A. H., *Astrophys. Jour.*, **95**, 356–385 (1942).

[3] Bowen, I. S., and Wyse, A. H., *Lick Obs. Bull.*, **19**, 1–16 (1939); Bowen, I. S., and Edlén, B., *Nature*, **143**, 374(1939).

[4] Spencer Jones, H., *Cape Obs. Ann.*, **10**, Part 9, 144 (1931).

[5] Private communication.

[6] Thesis, Harvard (1935).

[7] Menzel, D. H., Aller, L. H., and Hebb, M. H., *Astrophys. Jour.*, **93**, 230–235 (1941).

[8] Menzel, D. H., and Aller, L. H., *Ibid.*, **94**, 30–36 (1941).

[9] Payne-Gaposchkin, C., *Les Novae et les Naines Blanches*, Paris (1939).

[10] Payne-Gaposchkin, C., *Ann. Astrophys. Obs.* (Tonanzintla, Mex.),**1** (1942)(in press).

[11] Whipple, F. L., and Payne-Gaposchkin, C., *Harv. Obs. Circ.*, No. 413, 1–12 (1936).

[12] Menzel, D. H., and Aller, L. H., *Astrophys. Jour.*, **93**, 195–201 (1941).

[13] Menzel, D. H., and Aller, L. H., *Ibid.*, **93**, p. 199 (1941).

[14] Whipple, F. L., and Payne-Gaposchkin, C., *Harv. Obs. Circ.*, No. 413, p. 10 (1936).

[15] Krat, W., *Pulk. Obs. Circ.* No. 29, 21–25 (1940).

[16] Popper, D. M., *Publ. Astr. Soc. Pacific*, **53**, 252–255(1941).

ON THE DIMENSIONS AND CONSTITUTION OF VARIABLE STARS

BY CECILIA PAYNE-GAPOSCHKIN AND SERGEI GAPOSCHKIN

HARVARD COLLEGE OBSERVATORY

Communicated October 16, 1942

1. The Observational Material.—A unified study of the properties of variable stars has been carried out by the writers during the past few years.[1] With the accumulation of material relative to the periods, magnitudes and spectra of variable stars it is becoming possible not only to examine them as individuals, but to group them in families with common physical characteristics, and thus, perhaps, to approach the problems of their development and origin.

A general summary of what is now known or inferred concerning the principal physical parameters that describe the intrinsic variables—their luminosities, radii and masses—has been presented elsewhere by the authors.[2] References to the sources of the material are given in the paper cited. The principal results are shown graphically in figures 1 and 2. Figure 1 shows, on the left, the relation to the logarithm of the luminosity (in terms of the sun) to the logarithm of the period for Cepheid variables; on the right, the relationship of spectrum to logarithm of luminosity for the red variables, both semi-regular and long-period.

Figure 2 relates the sizes of Cepheid variables to their periods, and the

sizes of red variables to their spectral classes. The unit of size is the radius of the sun.

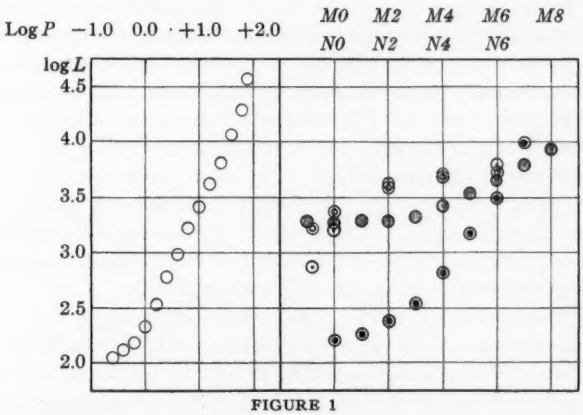

FIGURE 1

Luminosities of intrinsic variables as functions of period or spectrum. Ordinates are logarithms of luminosities in terms of the sun. The various types of variables are denoted by symbols as follows: Cepheid variables, circles; long-period *M* stars, shaded dots; long-period *N* stars, circles in circles; semi-regular *M* stars, large dots in circles; semi-regular *N* stars, small dots in circles.

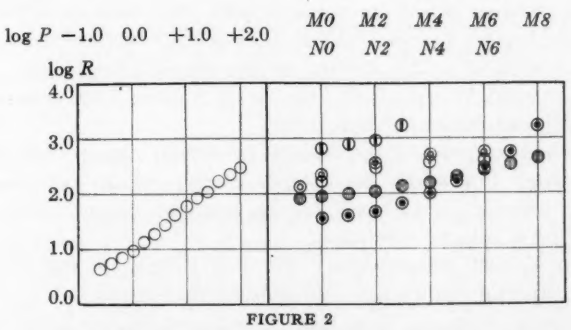

FIGURE 2

Sizes of intrinsic variables. Abscissae are logarithms of period (left) for Cepheids, spectra, for long-period and semi-regular variables. Ordinates are logarithms of radii, in terms of the solar radius. Symbols are as in figure 1. Barred circles refer to supergiant semi-regular variables.

It is seen from figures 1 and 2 that in luminosity and in size the Cepheids and the red variables greatly exceed the sun. Neither in size nor in luminosity, however, do the two groups constitute a continuous series. Per-

haps the supergiant red variables in figure 2 may be considered to follow from the Cepheids in dimensions.

2. The Constitution of Variable Stars.—Our knowledge of the internal conditions of the stars depends upon a knowledge of luminosity, mass, radius and molecular weight. In the calculations that will now be quoted, the mean molecular weight has been assumed to be 1.00, corresponding to a hydrogen content of 0.33. The central condensation (and, consequently, the polytropic index) has been calculated from the data of figures 1 and 2 by the methods of Chandrasekhar.[3] The mean polytropic indices thus obtained for the different groups of variables are as follows: Cepheid variables, 2.96; long-period variables, Class M, 3.15; long-period variables, Class N, 3.30; semi-regular variables, Class M, 3.19; semi-regular variables, Class N, 3.30, supergiant (semi-regular) M variables, 3.8: .

There is no evidence of high central condensation, nor do any of the intrinsic variables seem to deviate from polytropic index 3 in the direction of homogeneity. The calculations have assumed that the stars follow the (empirical) mass-luminosity relation. If they are under-luminous, the polytropic indices are smaller; if they are over-luminous, the polytropic indices are larger. We have, however, no evidence on departures of intrinsic variables from the mass-luminosity relation.

A second method of estimating the constitution of intrinsic variables arises from an application of the pulsation theory. It consists essentially in finding how the polytropic index must be adjusted to give the observed relationship between period and mean density. An estimate of the ratio of the specific heats is required for the application of this method: the value 1.429 was adopted. The average results are as follows: for Cepheids, 2.47; long-period M stars, 2.12; long-period N stars, 2.30; semi-regular M stars, 2.54; semi-regular N stars, 2.98.

The above estimates of the internal conditions suggest that the point-source model[4] (for which the exterior corresponds to polytropic index 3.25, the interior, to 1.5) will furnish a useful approximation for the examination of internal conditions.

3. The Central Temperatures.—For a star built on the point-source model, the central temperature T_c is given by the formula:[5]

$$\log T_c = 7.3175 + \log M - \log R,$$

where M and R are mass and radius in terms of those of the sun. For the standard model the temperature would be given by the formula:[6]

$$\log T_c = 7.2949 + \log \mu + \log \beta + \log M - \log R,$$

where μ and β are mean molecular weight (assumed equal to 1.00) and the ratio of radiation pressure to total pressure (appreciably less than unity for these high masses). The effect of the constant and the factor β is to

make the standard-model temperatures rather lower than the point-source model temperatures. The latter are indeed the *highest* that are compatible with the observational data.

Values of (log M − log R), and the corresponding temperatures (point-source model) are given in table 1. The relation of these central temperatures to period and spectrum for the variable stars concerned is shown in figure 3.

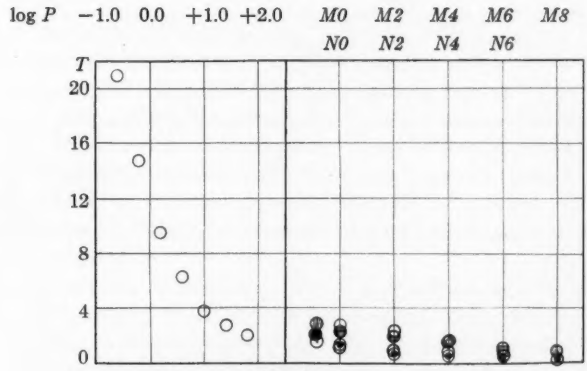

FIGURE 3

Central temperatures for different types of variable stars. Abscissae are as in figures 1 and 2. Ordinates are central temperatures in millions of degrees centigrade. Symbols are as in figures 1 and 2.

TABLE 1

CENTRAL TEMPERATURES FOR VARIABLE STARS

CEPHEID VARIABLES			LONG-PERIOD VARIABLES			SEMI-REGULAR VARIABLES		
LOG P	LOG M/R	LOG T_c	SPECTRUM	LOG M/R	LOG T_c	SPECTRUM	LOG M/R	LOG T_c
−0.6	0.00	7.32	*K5e*	−0.92	6.40	*M0*	−0.87	6.45
−0.2	−0.15	7.17	*M0e*	−0.95	6.37	*M2*	−0.95	6.37
0.2	−0.34	6.98	*M2e*	−1.02	6.30	*M4*	−1.12	6.20
0.4	−0.43	6.89	*M4e*	−1.13	6.19	*M6*	−1.37	5.95
0.6	−0.52	6.80	*M6e*	−1.30	6.02	*M8*	−1.74	5.58
0.8	−0.64	6.68	*M8e*	−1.43	5.89	*M9*	−1.93:	5.39:
1.0	−0.73	6.59	....	....	....	...	....	....
1.2	−0.79	6.53	*R8e*	−1.12	6.20	*R8*	−1.14	6.18
1.4	−0.88	6.44	*N0e*	−1.29	6.03	*N0*	−1.25	6.07
1.6	−0.96	6.36	*N2e*	−1.45	5.87	*N2*	−1.37	5.95
1.8	−1.00	6.32	*N4e*	−1.55	5.77	*N4*	−1.41	5.91
2.0	−1.06	6.26	*N6e*	−1.60	5.72	*N6*	−1.42	5.90

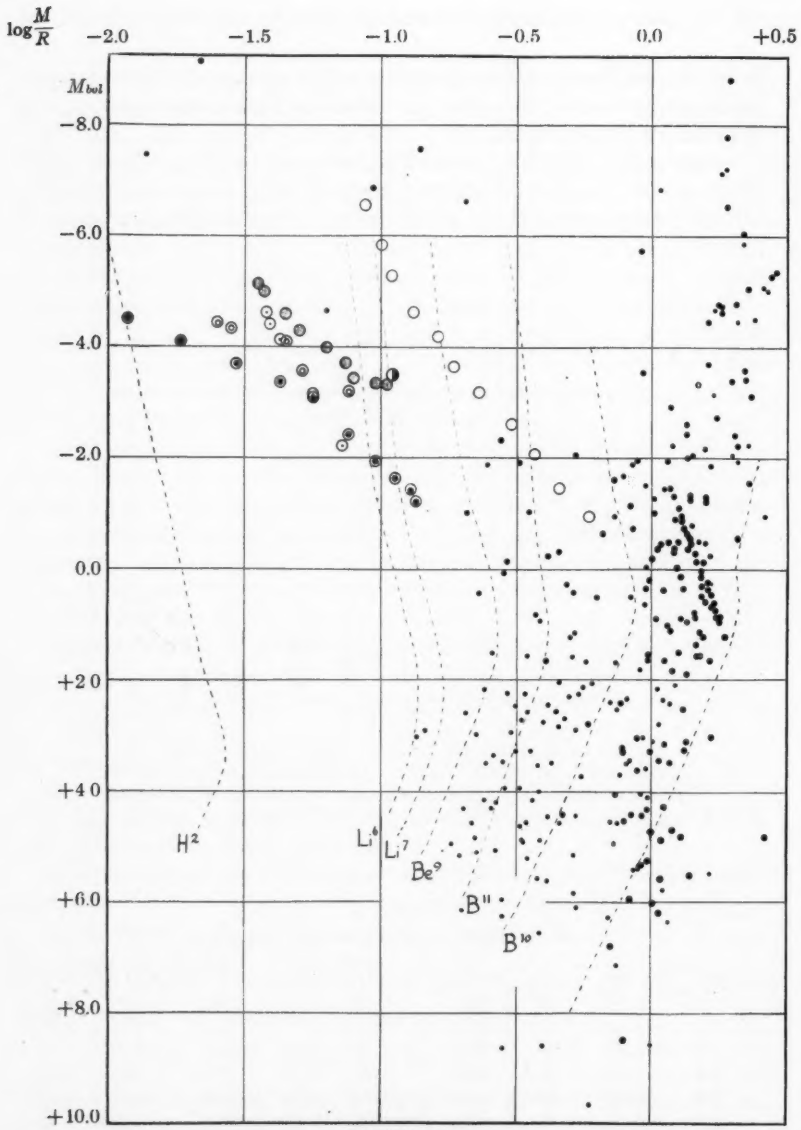

FIGURE 4

Relation between absolute bolometric magnitude (ordinate) and log M/R in solar units. The five groups of variable stars are denoted by symbols as in figures 1 to 3. The half-filled circle refers to RV Tauri stars. Smaller dots denote the first components of selected eclipsing variables; the smallest dots refer to the second components. Broken lines indicate the course of the subatomic reactions.

4. The Subatomic Processes.—The relationship, between central temperature of stars and the atomic processes that are the source of the radiated stellar energy, has been extensively discussed in recent years.[7] That the red variable stars probably utilize lighter elements than those used in the carbon cycle has been shown by Gamow and his associates.[8] The rôle played by the various subatomic processes is illustrated in figure 4, which presents the relationship between log M/R and absolute bolometric magnitude for the families of variable stars, and also for a number of other stars of well-known dimensions. Lines corresponding to the various subatomic processes are inserted in the figure.

Several suggestive conclusions can be drawn from figure 4. No stars fall in the region of deuterium as an energy source. Most of the long-period and semi-regular red variables fall in the lithium region. The Cepheids span the beryllium and boron regions, with a suggestion of transition from one subatomic process to another near the minima of period frequency. The point representing the RV Tauri stars[9] places them in such a region of transition, near the limit of the long-period M stars. The bright components of eclipsing stars provide a specimen of "normal" stars that fall in the region of the carbon cycle.

There are, however, a number of double star systems of which the two members fall in very different regions of the figure. That two members of one system are drawing on different energy sources is by no means a rare phenomenon, and raises provocative questions concerning the origin and evolution of such stellar pairs.

[1] Payne-Gaposchkin, C., *Amer. Phil. Soc. Proc.*, **81**, 189 (1939).

[2] Payne-Gaposchkin, C., and Gaposchkin, S., *Amer. Phil. Soc. Proc.* (1942) (in press.)

[3] Chandrasekhar, S., *Mon. Not., Roy. Astr. Soc.*, **96**, 647–660 (1936).

[4] Chandrasekhar, S., *An Introduction to the Study of Stellar Structure*, Chicago, p. 349 ff. (1938).

[5] Chandrasekhar, S., *Ibid.*, p. 354 (1939).

[6] Chandrasekhar, S., *Ibid.*, p. 230 (1939).

[7] Bethe, H., *Phys. Rev.*, **55**, 103 (1938); *Ibid.*, **55**, 434–456 (1939).

[8] Gamow, G., *Ibid.*, **55**, 718–725 (1939); Gamow, G., and Teller, E., *Ibid.*, **55**, 791 (1939); Greenfield, M., *Ibid.*, **60**, 175–183 (1941).

[9] Payne-Gaposchkin, C., Brenton, V. K., and Gaposchkin, S., *Harv. Obs. Ann.*, **113**, No. 1 (1942) (in press); Payne-Gaposchkin, C., and Brenton, V. K., these PROCEEDINGS, **28**, 496–500 (1942).

A STUDY OF THE RV TAURI VARIABLES

BY CECILIA PAYNE-GAPOSCHKIN AND VIRGINIA K. BRENTON

HARVARD COLLEGE OBSERVATORY

Communicated October 16, 1942

1. Definitions.—Among the intrinsic variables, the RV Tauri stars stand out with rather definite characteristics. Three principal criteria may be used for assigning stars to the class.

(*a*) Successive minima differ in depth. Deep and shallow minima are conventionally called "primary" and "secondary."

(*b*) Primary and secondary minima interchange phases at intervals.

(*c*) The stars are variable in spectrum and in radial velocity. The spectrum is of Class *F*, *G* or *K* at maximum.

The shortest known period is 38 days (EZ Aquilae), the longest is 146 days (R Scuti). Although the period usually assigned to these stars is that from one primary minimum to the next, a comparison with the velocity curve shows that the star undergoes a type of pulsation with one-half that period.

The brighter RV Tauri stars have recently been studied on the Harvard collection of stellar photographs. Of the twenty-nine stars now assigned to the class, fourteen proved to be bright enough for intensive study. A discussion of about sixteen thousand observations of these stars has been completed,[1] and the present note is intended to summarize some of the physical results.

2. The Secondary Characteristics.—Many RV Tauri stars show rather marked irregularities in period, and though over a long interval a mean period can always be assigned, instantaneous elements for short intervals may require periods differing by one or two per cent. This characteristic is not peculiar to RV Tauri stars: it is shared by other semi-regular variables and by long-period variables.

Many RV Tauri stars also show fluctuations of mean brightness; very marked for stars like DF Cygni and somewhat marked for AR Puppis (for both of which the fluctuation is periodic), marked, but not periodic for R Sagittae, and less conspicuous for U Monocerotis and R Scuti. On the whole, the fluctuations of mean brightness seem most marked for stars whose double period is near 75 days. For RV Tauri stars of longest period they are small. It may be noted on the other hand that interchanges of minima seem to be commonest for the stars of longest period; for stars like DF Cygni and AC Herculis they occur very rarely. Fluctuations of mean brightness are found for the AI Scorpii and μ Cephei stars, as well as for RV Tauri stars.

From comparison of the visual light curve with the photographic one, it

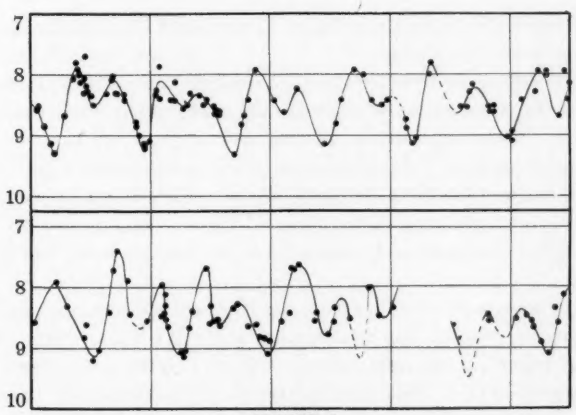

FIGURE 1

Variations of AC Herculis, a typical regular RV Tauri star. Ordinates are photographic magnitudes. Vertical lines are drawn at intervals of 100 days.

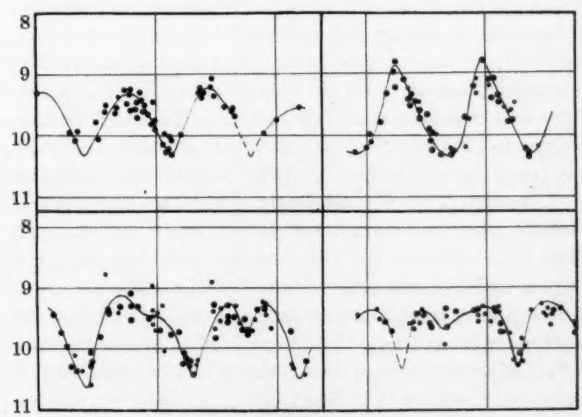

FIGURE 2

Variations of UU Herculis. Ordinates are photographic magnitudes. Vertical lines are drawn at intervals of 100 days. Above, the 72-day "fundamental" light curve. Below, the 45-day "overtone" light curve.

is evident that minima, both primary and secondary, occur somewhat earlier photographically. A rough measure of the difference in time indicates that on an average the photographic curve precedes the visual by

$0^P.026$. This value is twice as large as the shift that Kukarkin[2] found for the long-period Cepheids.

3. The Variations of UU Herculis.—Among the twenty-nine stars assigned to the RV Tauri group,[1] UU Herculis shows the characteristics only intermittently. The peculiarities of this star were pointed out by Gerasimovič some years ago.[3] Subsequent work has substantiated his conclusion that the star alternates between two periods: an RV Tauri-like curve shows a period of about 90 days, and a Cepheid-like curve, a period of 72 days. Three complete alternations between these two periods have been observed since 1900.

Figure 1 shows some excerpts from the Harvard photographic light curve of AC Herculis, perhaps the most regular of the RV Tauri stars. It shows the typical aspect of the light curve. Figure 2 shows some excerpts from the variations of UU Herculis: above is the Cepheid-like light curve; below, the RV Tauri-like curve. The ratio between the physically significant half-period of the RV Tauri-like curve and the period of the Cepheid-like curve is 0.63. Both periods can be represented by linear elements throughout the observed interval.

Gerasimovič expressed the conviction that the two periods, though not in an obvious, simple ratio, could not be physically independent. More recently it has been shown by Schwarzschild[4] that both overtone and fundamental pulsations may be found among the pulsating stars of short period He has calculated that the ratio between the fundamental period and the period of the first overtone should be between 0.55 and 0.74, according as the ratio of specific heats of the material within the star is between 1.43 and 1.67. It is tempting to conclude that the Cepheid-like light curve represents the fundamental for UU Herculis, while the RV Tauri-like curve (with, of course, half the conventional period) represents the overtone pulsation. The ratio between the two periods, 0.63, falls in the middle of the range given by Schwarzschild. If the RV Tauri-like variations of these stars with alternating minima are in fact overtone pulsations, the conventional period must be multiplied by about 7/20 to obtain the fundamental period that is physically comparable to the period of a Cepheid variable.

4. Relationships between RV Tauri Stars and Other Variables.—The periods of RV Tauri stars fall between those of Cepheids and long-period variables, with a certain amount of overlapping at both ends. They have usually been regarded as transition objects whose irregularities are related to a minimum of period frequency.

The relationship between the period and spectra of the RV Tauri stars, and the comparison with other intrinsic variables give a picture of the rôle that they play among the variable stars. The material is shown in figure 3, for which the data have been taken from the study cited earlier.[1] Logarithms of period are plotted against spectrum, and in addition to RV

Tauri stars, Cepheids, semi-regular yellow and red variables, and long-period variables are shown.

A striking feature of figure 3 is the large differences of spectrum for RV Tauri stars of given period. The spectra of these stars are notoriously

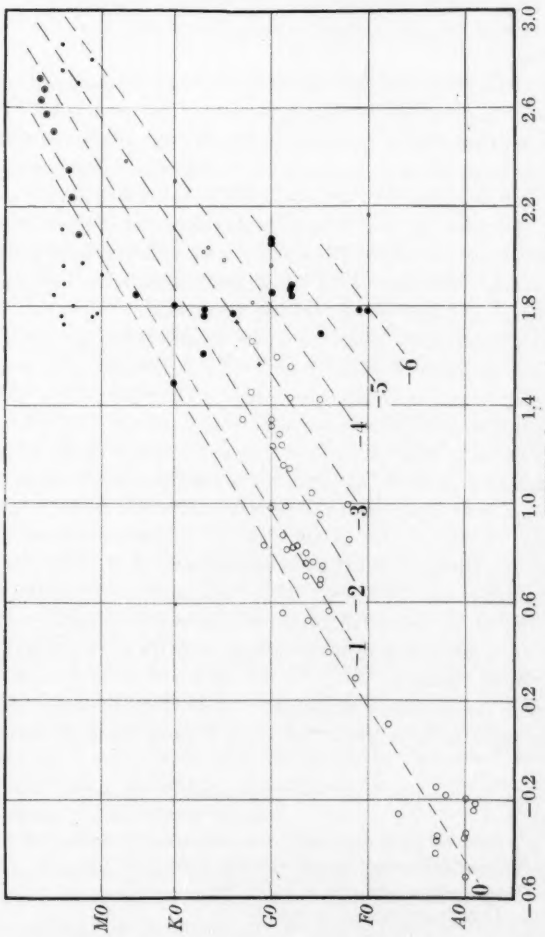

FIGURE 3

Logarithm of the period (abscissa) and maximal spectrum for intrinsic variable stars. Large dots denote RV Tauri stars, large circles, Cepheid variables; small circles, semi-regular yellow variables; small dots, semi-regular red variables; dots in circles, long-period variables (average period-spectrum relation). Broken lines denote lines of equal absolute photographic magnitude.

difficult to classify, but that AC Herculis and AR Puppis are early F stars, and DF Cygni and V Vulpeculae late G stars, are well-substantiated observations. A smaller, but quite definite, dispersion in the period-spectrum relation for Cepheids has been well known for some time.

A number of lines have been inserted in figure 3 to show the probable course of lines of equal absolute photographic magnitude. The lines have been defined at one end by means of the period-luminosity relation and the period-spectrum relation[5] (relating to maximum light,[6] for the median spectra of RV Tauri stars are deceptive). At the upper end they have been drawn with the aid of Keenan's study[7] of the luminosities of the small-range red variables.

If the course of the lines of equal absolute photographic magnitude be accepted, it is seen that the period-luminosity relation for the RV Tauri stars is so widely scattered as to be non-existent, though the *mean* relation would be nearly continuous with that for the Cepheids. The latter, and the semi-regular yellow variables that are not RV Tauri stars (such as AG Aurigae and SX Herculis) seem to pass through the center of the RV Tauri stars, which now appear as Cepheid-like stars of exceptional color for their periods. Very possibly the class of RV Tauri stars, marked by light curves of exceptional form, is not a physically homogeneous one.

Two stars that are certainly allied to, if not members of, the RV Tauri group, are inserted in figure 3 with crosses. These are the semi-regular variables in Messier 4[8] and in Omega Centauri.[9] They have been plotted with respect to *absolute photographic magnitude* and period. Clearly they fit closely among the RV Tauri stars, and make it seem probable that the lines of equal absolute magnitude are drawn in a roughly correct manner.

Figure 3 throws a little light upon the period-luminosity relationship for Cepheid variables. If the course of the lines in the figure is correct, it would follow that at a given period there is a dispersion of ± 0.5 in absolute photographic magnitude for Cepheids. This value may be compared with the observed scatter of the Magellanic period-luminosity relation,[10] which very likely refers to a more homogeneous group of stars than the galactic specimens collected in figure 3.

The photographic observations of the RV Tauri stars discussed in this paper were made possible by a grant from the Milton Fund of Harvard University.

[1] Payne-Gaposchkin, C., Brenton, V. K., and Gaposchkin, S., *Harv. Obs. Ann.*, **113** No. 1, 1942 (in press.)

[2] Kukarkin, B. W., *Nijni Novgorod Var. Stars, Nijni Novgorod*, **5**, 42–45 (1936).

[3] Gerasimovič, B. P., *Harv. Obs. Bul.*, No. 857, 27–32 (1928).

[4] Schwarzschild, M., *Astrophys. Jour.*, **94**, 245–252 (1941).

[5] Shapley, H., these PROCEEDINGS, **26**, 541–548 (1940).

[6] Payne-Gaposchkin, C., and Gaposchkin, S., *Variable Stars*, Cambridge, p. 155, (1938).

[7] Keenan, P. C., *Astrophys. Jour.*, **95**, 461–467 (1942).

[8] Sawyer, H. B., *Jour. Roy. Astr. Soc. Can.*, **36**, 213–217 (1942).

[9] Martin, W. C., *Bul. Astr. Netherlands*, **8**, 290–292 (1938).

[10] Shapley, H., McKibben, V., and Craig, R. A., these PROCEEDINGS, **28**, 192–199 (1942).

GALACTIC AND EXTRAGALACTIC STUDIES, XVI. PHOTOGRAPHIC AMPLITUDES OF CLASSICAL CEPHEIDS

BY HARLOW SHAPLEY

HARVARD COLLEGE OBSERVATORY

Communicated October 13, 1942

1. The correlation of the amplitude of variation with period length and with absolute magnitude for classical Cepheid variables is derived with uncertainty from existing data for the galactic Cepheids because of the following difficulties:

(*a*) The systems of photographic magnitudes used by the various workers depend on different telescopes and photographic emulsions; they have not been and cannot well be brought to a single uniform standard;

(*b*) The comparison star sequences vary in security;

(*c*) The colors of Cepheids are too poorly known to permit dependable reduction of visual light curves and amplitudes to the photographic system;

(*d*) There may be an uneven but distinct selection of stars of large range by some observers.

For 86 variables of the galactic system discussed by Ludendorff[1] we find a mean amplitude of 1.23. For 232 variables in Theile's compilation[2] we compute a mean photographic amplitude of 1.22.

2. The study of the Cepheid variable stars in the Small Magellanic Cloud has provided material that is uncommonly homogeneous with respect to magnitude system, and therefore suitable for the correlation of amplitude with period and with the median apparent magnitude. All the sequences and all the measures have been reduced to a single sequence and a single observer. Moreover, the number of variables is so large that the correlation can be examined separately for the different parts of the Cloud.

In an earlier paper[3] we have summarized the amplitudes for the classical Cepheids in the Small Cloud, and noted that the background of faint stars in the denser parts of the nucleus diminishes the observable amplitudes on the average by a seventh of a magnitude. Elsewhere in the Cloud the "doubling" effect can be ignored.

3. In table 1, the amplitudes are grouped into means for the 562 Cepheid variables in the Small Cloud—all those for which periods and light curves had been derived from Harvard plates at the time of this compilation. The results are plotted in figure 1. The vertical line through each plotted point has a length double the corresponding mean error of the mean amplitude. The larger amplitudes for the variables with periods in excess of 10 or 12 days have long been known from the earlier evidence from the Magellanic Clouds as well as from the Galactic System. It is verified for galactic Cepheids in Theile's[2] analysis, which is represented also in figure 1 by the

plotted crosses and the broken lines. It seems unlikely, however, that the amplitudes for galactic Cepheids are systematically greater than that for

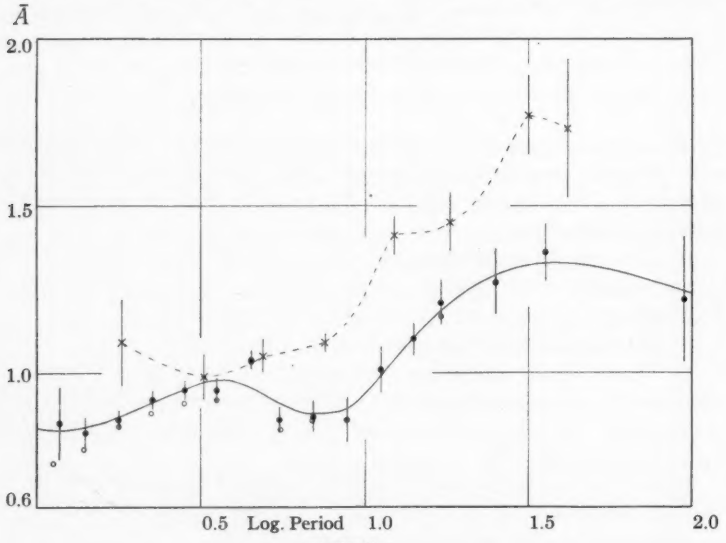

FIGURE 1

Mean amplitudes and logarithms of periods for Small Magellanic Cloud (dots) and Galaxy (crosses, Theile).

TABLE 1

PERIOD AND AMPLITUDE

INTERVAL OF LOG. PERIOD	MEAN LOG. PERIOD		MEAN AMPLITUDE		MEAN ERROR	NUMBER OF VARIABLES
			m	m		
0.0–0.1	0.071	(0.051)	0.85	(0.73)	±0.11	5
0.1–0.2	0.148	(0.145)	0.82	(0.77)	0.05	37
0.2–0.3	0.252	(0.251)	0.86	(0.84)	0.03	67
0.3–0.4	0.355	(0.349)	0.92	(0.88)	0.03	58
0.4–0.5	0.454	(0.451)	0.95	(0.91)	0.03	90
0.5–0.6	0.549	(0.549)	0.95	(0.92)	0.04	69
0.6–0.7	0.656	(0.655)	1.04	(1.04)	0.03	53
0.7–0.8	0.740	(0.742)	0.86	(0.83)	0.04	34
0.8–0.9	0.844	(0.842)	0.87	(0.86)	0.05	33
0.9–1.0	0.947	...	0.86	..	0.07	24
1.0–1.1	1.051	...	1.01	..	0.07	21
1.1–1.2	1.149	...	1.10	..	0.05	23
1.2–1.3	1.233	(1.233)	1.21	(1.17)	0.07	16
1.3–1.5	1.401	...	1.27	..	0.10	15
1.5–1.7	1.554	...	1.36	..	0.09	11
>1.7	1.978	...	1.22	..	±0.19	6

the Magellanic Cloud by the amount indicated by Theile's results. The matter merits further investigation, not only in the study of the light curves

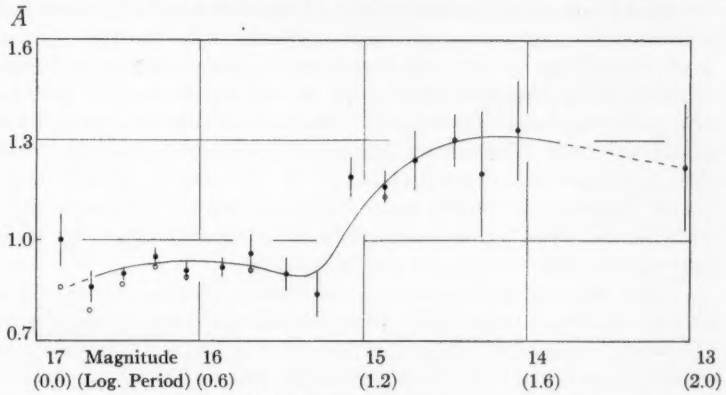

FIGURE 2

Luminosity-amplitude relation for 562 Cepheids in the Small Cloud. Ordinates are mean amplitudes.

TABLE 2

LUMINOSITY AND AMPLITUDE

INTERVAL OF APP. MAGNITUDE	MEAN MAGNITUDE		MEAN AMPLITUDE		MEAN ERROR	NUMBER OF VARIABLES
	m	*m*	*m*	*m*		
17.00–16.80	16.85	(16.85)	1.00	(0.86)	±0.08	18
16.79–16.60	16.66	(16.67)	0.86	(0.79)	0.05	45
16.59–16.40	16.46	(16.47)	0.90	(0.87)	0.02	92
16.39–16.20	16.27	(16.27)	0.95	(0.92)	0.03	96
16.19–16.00	16.08	(16.08)	0.91	(0.89)	0.03	86
15.99–15.80	15.86	...	0.92	..	0.03	63
15.79–15.60	15.69	(15.69)	0.96	(0.91)	0.06	30
15.59–15.40	15.47	...	0.90	..	0.05	40
15.39–15.20	15.28	...	0.84	..	0.07	17
15.19–15.00	15.08	...	1.19	..	0.06	20
14.99–14.80	14.87	(14.87)	1.16	(1.13)	0.05	16
14.79–14.60	14.69	...	1.24	..	0.09	8
14.59–14.40	14.45	...	1.30	..	0.08	10
14.39–14.20	14.28	...	1.20	..	0.19	7
14.19–14.00	14.06	...	1.33	..	0.15	8
]14.00	13.03	...	1.22	..	±0.19	6

of galactic Cepheids but also through further check of the magnitude system of the Small Cloud.

The reality, for the Small Cloud variables, of the preliminary maximum in the period-amplitude curve for periods between 2.5 and 5 days is not very

certainly established, notwithstanding the indicated small mean errors in the mean amplitudes.

In the determination of the periods and light curves of the 562 Cepheids used in table 1, and in the next section, 85 other variables were measured on all available plates, and preliminary median magnitudes and amplitudes are available, although the periods can as yet only be roughly estimated from the period-luminosity relation. The periods have often been difficult to find because of the small amplitudes. It is therefore appropriate to include these provisional results in a study of the period-amplitude relation and the luminosity-amplitude relation discussed below. The parenthetical entries in the tables, and the open circles in the figures, refer to the revised means that include the 85 "unsolved" Cepheids.

4. The high correlation between the absolute magnitudes and the periods for classical Cepheids (the period-luminosity relation) suggests that a luminosity-amplitude relation should be closely similar to the period-amplitude relation. In table 2 and figure 2 the data for the 562 Cepheids are reassembled to examine this point. The question of the correctness of the magnitude system in the Small Cloud is, of course, also involved in the luminosity-amplitude relation. Assuming its reliability, we find that the amplitudes are, as expected, systematically smaller for the absolutely fainter variables. When the median luminosities exceed absolute magnitude -2.2, corresponding to apparent magnitudes brighter than 15.15 and periods greater than 13 days, the mean amplitude averages $1^m.22$, while for fainter classical Cepheids it is three-tenths of a magnitude less. The brighter Cepheids are redder, but no reliable data are available to test the probable connection between amplitude and color, that is, between amplitude and the wave-length of the maximum in the spectrum-energy curve. The bolometric amplitudes would likely show a somewhat different relation with period and luminosity.

5. Only about one-third of the known variables in the Small Cloud have been measured for amplitude and period. The possibility that the systematic selection of large ranges, or of periods of convenient length, has affected the results summarized in table 1 and figure 1 leads to the necessity of examining the correlation on the basis of the material that is available from those regions of the Cloud that have been thoroughly studied. Table 3 and figure 3 present the results for the 365 variables located in such regions. The relation of mean amplitude to period is very similar to that found for the whole Cloud. Again the suspected Cepheids of unmeasured period are included to form the parenthetical means, which are again plotted as open circles.

Since these data appear to be free of selection, at least for periods greater than two days, we should adopt table 3 and figure 3 as best representing the correlation in the Small Magellanic Cloud.

6. Recognizing the danger of subdividing the observational material too much, we give, nevertheless, in table 4, the mean amplitudes separately for the nuclear, intermediate and border regions of the Cloud, and in each for three intervals of period length. Only the material (365 variables) from the completely surveyed regions is used.

Small amplitudes are found for short period variables in the border regions—possibly an effect on measured magnitudes of distance from plate center. The larger ranges for the longest periods in the same regions may

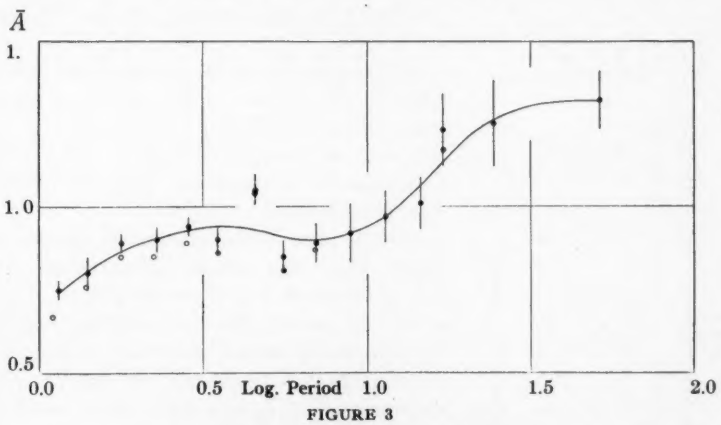

FIGURE 3

Amplitudes and periods for special regions.

TABLE 3

PERIOD AND AMPLITUDE IN FULLY EXPLORED REGIONS

INTERVAL OF LOG. PERIOD	MEAN LOG. PERIOD		MEAN AMPLITUDE		MEAN ERROR	NUMBER OF VARIABLES
			m	m	m	
0.0–0.1	0.059	(0.043)	0.75	(0.67)	±0.03	3
0.1–0.2	0.149	(0.145)	0.80	(0.76)	0.05	29
0.2–0.3	0.252	(0.251)	0.89	(0.85)	0.03	46
0.3–0.4	0.360	(0.351)	0.90	(0.85)	0.04	40
0.4–0.5	0.456	(0.451)	0.94	(0.89)	0.03	63
0.5–0.6	0.545	(0.546)	0.90	(0.86)	0.04	43
0.6–0.7	0.659	(0.657)	1.05	(1.04)	0.05	28
0.7–0.8	0.745	(0.747)	0.85	(0.81)	0.05	21
0.8–0.9	0.845	(0.843)	0.89	(0.87)	0.06	23
0.9–1.0	0.949	...	0.92	..	0.09	18
1.0–1.1	1.055	...	0.97	..	0.08	13
1.1–1.2	1.164	...	1.01	..	0.08	14
1.2–1.3	1.233	(1.233)	1.23	(1.17)	0.11	9
1.3–1.5	1.386	...	1.25	..	0.13	10
>1.5	1.712	...	1.32	..	±0.09	5

TABLE 4

MEAN PERIOD AND AMPLITUDE IN VARIOUS REGIONS[a]

INTERVAL OF LOG. PERIOD	NUCLEUS			INTERMEDIATE			BORDERS			ALL	
	NO.	$\bar{A}$	M. E.	NO.	$\bar{A}$	M. E.	NO.	$\bar{A}$	M. E.	NO.	$\bar{A}$	M. E.
<0.4	25	0.91	±0.05	53	0.91	±0.03	40	0.80	±0.03	118	0.87	±0.02
0.4–0.8	36	0.94	±0.04	75	0.93	±0.03	44	0.95	±0.05	155	0.94	±0.02
>0.8	36	0.95	±0.05	19	1.01	±0.07	37	1.13	±0.06	92	1.03	±0.04
All	97	0.93	±0.03	147	0.93	±0.02	121	0.95	±0.03	365	0.94	±0.01

[a] This tabulation does not include the 85 suspected Cepheids in the regions.

perhaps owe something to the same effect. The point can be readily checked by using series of plates appropriately centered; such a series will be made at the Boyden Station.

The mean amplitude for all 365 variables is $0^m.94$, a value so low compared with the values derived for galactic Cepheids, $1^m.22$, that, as intimated above, the presence of systematic errors must be suspected. They might be attributed to one or more of the following sources:

(*a*) Background effect in the Small Cloud (see fourteenth paper of this series). This appears to be excluded by the evidence of the means in the last line of table 4; the average amplitude in the crowded nucleus is not less than elsewhere.

(*b*) The magnitude system in the Cloud, which has been made homogeneous, and checked for scale as well as zero point. A new observational program is in progress, designed to connect the master magnitude sequence of the Magellanic Clouds with Selected Area 94 at declination 0° and, with the coöperation of Dr. Baade of Mount Wilson, with the International magnitude standards at the North Pole.

(*c*) Length of photographic exposure. This factor could affect the amplitude of only the shortest periods, since the exposures on the plates used at Harvard for the Magellanic Clouds very rarely exceed one hour.

(*d*) Incompleteness of light curves for galactic variables, which generally leads to estimating amplitudes from the extreme maximum and minimum observations and not from the more trustworthy mean light curves.

(*e*) Untrustworthy magnitude sequences, preferential selection of large-range variables, and general non-homogeneity of observations for galactic Cepheids, as mentioned in section 1 above.

The problem is worth further serious consideration because of the persistent question

as to the identity of the laws of Cepheid variation from one galaxy to the next.

Finally it should be noted that for any given period and absolute magnitude there is a large dispersion in the amplitudes of classical Cepheids—a dispersion that far exceeds the observational uncertainties. Ranges of 0.5 and 1.5 magnitudes occur all along the period-amplitude curve. In this paper we have been concerned only with the mean amplitudes, and our conclusions refer only to the general tendency of amplitudes to depend on length of period or on absolute luminosity.

[1] *Sitzber. preuss. Akad. Wiss., Physik-math. Klasse*, **5**, 75–78 (1939).
[2] *Veröff. Berlin-Babelsberg*, **12**, III, 36 (1939).
[3] These PROCEEDINGS, **28**, 195, 197 (1942); the fourteenth paper of this series.